Dissertation on

ROLE OF INTRASTROMAL VORICONAZOLE

IN FUNGAL KERATITIS RESISTANT TO TOPICAL ANTIFUNGALS

Submitted in partial fulfillment of requirements of

M.S. OPHTHALMOLOGY

BRANCH - III

REGIONAL INSTITUTE OF OPHTHALMOLOGY

MADRAS MEDICAL COLLEGE

CHENNAI- 600 003

THE TAMILNADU

DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL 2015

CERTIFICATE

This is to certify that this dissertation entitled **"**ROLE OF INTRASTROMAL VORICONAZOLE IN FUNGAL KERATITIS RESISTANT TO TOPICAL ANTIFUNGALS **"** is a bonafide record of the research work done by **Dr. M.MUTHUMEENA,** post graduate in Regional Institute of Ophthalmology and Government Ophthalmic Hospital, Madras Medical College and Government General Hospital, Chennai-03, in partial fulfillment of the regulations laid down by The Tamil Nadu Dr. M.G.R. Medical University for the award of M.S. Ophthalmology Branch III, under my guidance and supervision during the academic years 2012-2015.

Prof. Dr.M.ANANDA BABU
CHIEF
RIO – GOH
Egmore, Chennai – 08

PROF.Dr.K.NAMITHA BHUVANESWARI, MS.,DO.
Director and Superintendent
RIO – GOH
Egmore, Chennai – 08

Prof. Dr. R.VIMALA, M.D
Dean,
Madras Medical College.
and Government General Hospital
Chennai **–**03

ACKNOWLEDGEMENT

I express my sincere thanks and gratitude to **Prof. Dr. R.VIMALA, M.D,** Dean, Madras Medical College and Government General Hospital for permitting me to conduct this study.

I have great pleasure in thanking **Prof.Dr.K.NAMITHA BHUVANESWARI, M.S ,D.O,** Director and Superintendent, RIO – GOH, Madras Medical College, for her valuable advice in preparing this dissertation.

I express my profound gratitude to **Prof. Dr.M.ANANDA BABU, M.S., DO. ,** my unit chief and my guide for his valuable guidance and constant support at every stage throughout the period of this study.

I am very grateful to my Co-Guides **Dr. V.SHARMILA DEVI M.S., Dr. K.S.T LATHA M.S. ,** for rendering their valuable advice and guidance for the study.

I wish to express my sincere thanks to all the professors, assistant professors and all my colleagues who had helped me in bringing out this study.

Finally, I am indebted to all the patients for their sincere co-operation for the completion of this study.

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. M. Muthumeena,
Postgraduate
RIOGOH,
Madras Medical College, Chennai-3.

Dear Dr. M. Muthumeena,

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Role of Intrastromal Voriconazole in Fungal Corneal Ulcers resistant to topical Antifungals"** No.28062014

The following members of Ethics Committee were present in the meeting held on 03.06.2014 conducted at Madras Medical College, Chennai-3.

1.	Dr. C. Rajendran, M.D.	-- Chairperson
2.	Dr. R. Vimala, M.D., Dean, MMC, Ch-3.	-- Deputy Chair Person
3.	Prof. Kalaiselvi, MD., Vice-Principal, MMC, Ch-3	-- Member
4.	Prof. Nandhini, M.D. Inst. of Pharmacology, MMC, Ch-3.	-- Member
5.	Dr. G. Muralidharan, Director Incharge , Inst. of Surgery	-- Member
6.	Prof. Md Ali, MD., DM., Prof & HOD of MGE, MMC, Ch-3.	-- Member
7.	Prof. Ramadevi, Director i/c, Biochemistry, MMC,Ch-3.	-- Member
8.	Prof. Saraswathy, MD., Director, Pathology, MMC, Ch-3.	-- Member
9.	Prof. Tito, Director, i/c. Inst. of Internal Medicine, MMC	-- Member
10.	Thiru. Rameshkumar, Administrative Officer	-- Lay Person
11.	Thiru. S. Govindasamy, BABL, High Court, Chennai-1.	-- Lawyer
12.	Tmt. Arnold Saulina, MA MSW	-- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

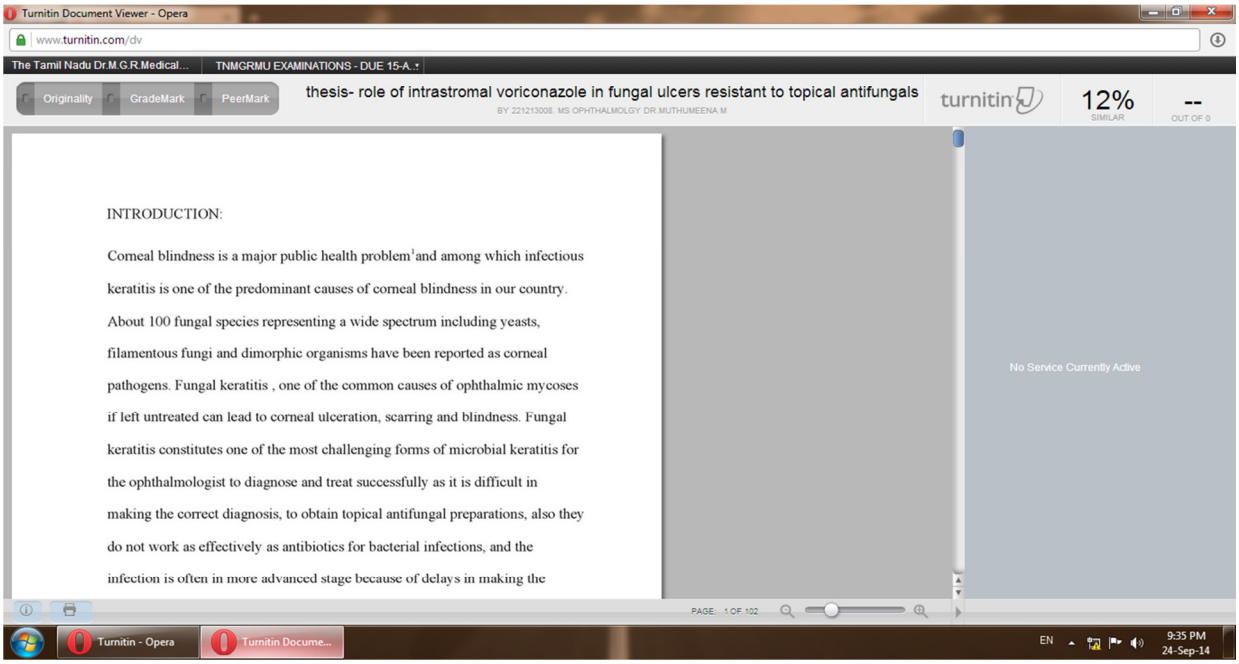
The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information, informed consent and asks to be provided a copy of the final report.

MADRAS MEDICAL COLL... CHENNAI-600 003
- 4 JUN 2014

4/6/14

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003


Turnitin Document Viewer - Opera
www.turnitin.com/dv
The Tamil Nadu Dr.M.G.R.Medical...
TNMGRMU EXAMINATIONS - DUE 15-A...
Originality
GradeMark
PeerMark
thesis- role of intrastromal voriconazole in fungal ulcers resistant to topical antifungals
BY 221213008. MS OPHTHALMOLGY DR.MUTHUMEENA M
turnitin
12%
SIMILAR
--
OUT OF 0
INTRODUCTION:
Corneal blindness is a major public health problem[1]and among which infectious keratitis is one of the predominant causes of corneal blindness in our country. About 100 fungal species representing a wide spectrum including yeasts, filamentous fungi and dimorphic organisms have been reported as corneal pathogens. Fungal keratitis , one of the common causes of ophthalmic mycoses if left untreated can lead to corneal ulceration, scarring and blindness. Fungal keratitis constitutes one of the most challenging forms of microbial keratitis for the ophthalmologist to diagnose and treat successfully as it is difficult in making the correct diagnosis, to obtain topical antifungal preparations, also they do not work as effectively as antibiotics for bacterial infections, and the infection is often in more advanced stage because of delays in making the
No Service Currently Active
PAGE: 1 OF 102
Turnitin - Opera
Turnitin Docume...
EN
9:35 PM
24-Sep-14

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "**A STUDY ON ROLE OF INTRASTROMAL VORICONAZOLE IN FUNGAL KERATITIS RESISTANT TO TOPICAL ANTIFUNGALS** " is a bonafide and genuine research work carried out by me under the guidance of PROF.DR.M.ANANDA BABU**.**

DATE:

PLACE: DR.MUTHUMEENA.M

ABBREVIATIONS

KOH- 10% POTASSIUM HYDROXIDE MOUNT

TKP- THERAPEUTIC PENETRATING KERATOPLASTY

Asp. Niger- aspergillus niger

Asp.Flavus- aspergillus flavus

Asp.fumigatus- aspergillus fumigates

RE- RIGHT EYE

LE- LEFT EYE

PAS- Periodic acid Schiff stain

CONTENTS

PART – II

ANNEXURE

PART – III

1. PROFORMA
2. MASTER CHART
3. BIBLIOGRAPHY
4. KEYWORDS

PART-1

INTRODUCTION:

Corneal blindness is a major public health problem [1]and among which infectious keratitis is one of the predominant causes of corneal blindness in our country. About 100 fungal species representing a wide spectrum including yeasts, filamentous fungi and dimorphic organisms have been reported as corneal pathogens. Fungal keratitis , one of the common causes of ophthalmic mycoses if left untreated can lead to corneal scarring and blindness. Fungal keratitis constitutes one of the most challenging forms of microbial keratitis for the ophthalmologist to diagnose and treat successfully as it is difficult in making the correct diagnosis, to obtain topical antifungal preparations, also they do not work as effectively as antibiotics for bacterial infections, and the infection is often in more advanced stage because of delays in making the correct diagnosis[3]. There may be therefore limited success in treatment. The main limitations of antifungal treatment include narrow antifungal spectrum of activity, poor tissue penetration, significant side effects or fungal resistance. To overcome these problems alternative routes such as intracameral & intrastromal routes are being evaluated. Intervention with intrastromal voriconazole has been found to be effective in cases of fungal ulcers not responding to topical antifungals in this study.

REVIEW OF LITERATURE:

The cornea is a transparent avascular watch glass like structure comprising the anterior one sixth of the outer coat of the eyeball

FUNCTIONS OF CORNEA:

Cornea is a highly specialized structure which refracts and transmits light which is necessary for ideal visual function. It subserves the following functions:

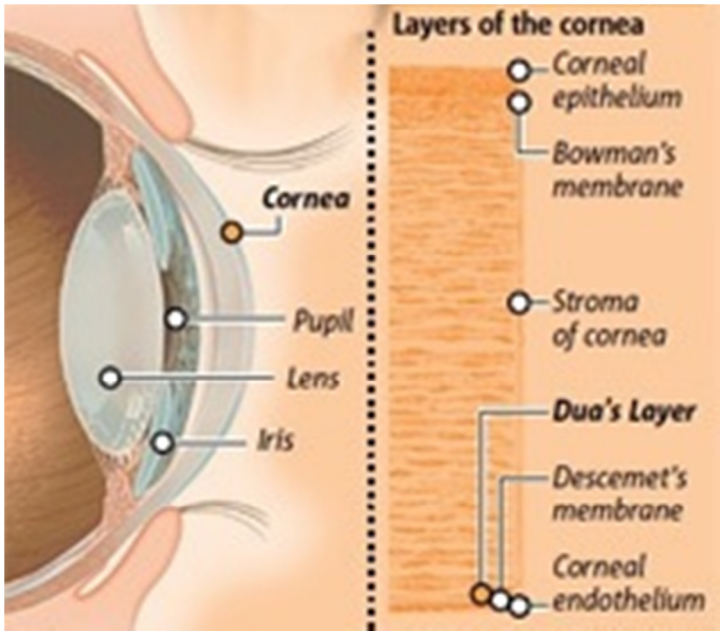
- Forms a protective interface with the environment
- Refracts light. 70% of the total refractive power is contributed by the cornea.
- Contains the intraocular pressure[1]

ANATOMY OF CORNEA:

The anterior surface of the cornea is elliptical while the posterior surface is circular with the former having average horizontal diameter of 11.75 mm, vertical diameter of 11 mm and the latter's average diameter being 11 mm.[8] Cornea is approximately 1- mm thick peripherally and 0.5 mm thick centrally.[5]

It comprises of five layers: epithelium, bowmans layer, stroma, descemets membrane and endothelium. Recently in 2013 harminder singh dua et al suggested

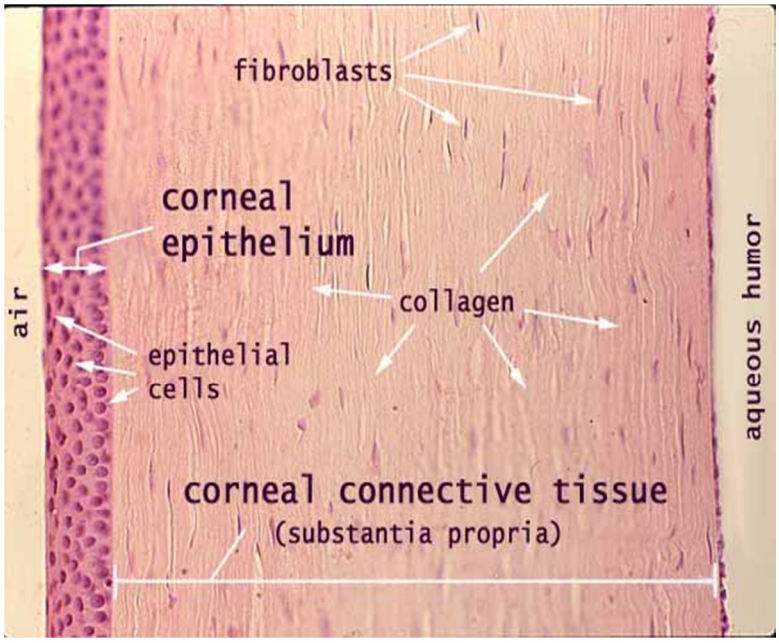
the existence of another layer called Dua's layer between the stroma and the descemets membrane.[1]



1.EPITHELIUM:The stratified squamous epithelium has about five to seven layers is around 50 microns thick. It has three to four outer flat squamous cells, one to three middle layer of epithelial cells called wing cells as they have lateral wing like extensions and single basal layer of columnar cells.[4] The complete turnover of the epithelium takes place in approximately five to seven days. Keratins constitute the major protein of the corneal epithelium. The basal cells of the epithelium adheres to the basement membrane and underlying stroma through adhesion molecules.

2.Bowman's layer is an acellular layer composed of proteoglycans and collagen fibrils. It shows greater resistance to both injuries and infections. It does not regenerate once destroyed.

3.The stromal layer is approximately 500 micron thick and forms about 90% of the cornea. It is secreted and maintained by the stromal fibroblasts termed keratocytes. Birk and Trelstad demonstrated that stromal surface compartments with bundles of parallel collagen fibrils are present in fibroblasts.



4.Descemet's membrane is the basement membrane of the corneal endothelium and is synthesized by the latter. At birth it is approximately 3 microns wide but by late adulthood it can increase upto 12 microns. It can regenerate when destroyed.in the periphery it ends as schwalbe's line at the anterior limit of the trabecular

meshwork.on electron microscopy it has vertically banded anterior one third region and granular amorphous posterior two third region. [2]

5. The corneal endothelium is a single layered low cuboidal epithelium. There are large numbers of mitochondria within the cytoplasm of endothelial cells. It does not regenerate unlike the epithelium. It is metabolically active. In the basolateral border of the endothelial cells Na-K-activated adenosine triphosphatase pump is present which plays a major role in maintaining stromal hydration. The endothelial cells are attached by desmosomes to the descemets membrane and laterally to each other by tight junctional complexes.

BLOOD SUPPLY: Cornea is an avascular structure. It derives its nourishment from small loops in the sub conjunctival space derived from the anterior ciliary vessels which invade its periphery . however corneal vascularisation can occur in various disease processes the purpose of which is to facilitate transport of systemic antibiotics and nutrition. Maurice et al demonstrated that both mechanical-structural loosening of stroma by edema and chemical- vasostimulatory factor are essential for neovascularisation to occur. [6]

CORNEAL INNERVATION: The corneal epithelium is the most highly innervated of all epithelia. the sub basal plexus forms a dense meshwork over a large central area. Muller et al studied that there are about 6000 nerve bundles in

the sub basal corneal plexus comprising of unmyelinated straight and beaded nerve fibre bundles each of which gives rise to seven axons resulting in 19000 to 44000 axons. [6] These give off about twenty nerve terminals which are extrapolated to result in around 7000 nociceptors/mm2 therby making cornea as the most highly innervated structure in the body. There are however no nerves in the central posterior part of the cornea, descemets membrane and the endothelium. The existence of these nerves is crucial for the health of the corneal tissue. Within a day of corneal nerve impairment epithelial cells lose their microvilli by swelling up and slough off at an accelerated rate. Therefore the epithelium loses its ability to heal after injury and even if healed the new tissue will be at a higher risk of spontaneous breakdown after corneal denervation.

APPLIED PHYSIOLOGY OF CORNEA:

Its two primary physiological functions are that it acts as a powerful refractive medium and it protects the intraocular contents.

The factors contributing to corneal transparency include anatomical factors such as avascularity, absence of myelination in nerves, uniform and regular arrangement of corneal epithelium and the peculiar arrangement of corneal lamellae and physiological factors i.e cornea being in a relative state of dehydration.

Maurice proposed the lattice theory which states that: the corneal transparency is maintained as the stromal collagen fibrils are equidistant from each other and are of equal diameter(275-350 A). this arrangement causes the cancellation of scattering of incident ray by each collagen fibril by the interference of other scattered ray.[3]

The factors affecting the corneal hydration include: the corneal epithelial and endothelial barrier, intraocular pressure and the metabolic pump function. Compromise of either of these barriers results in corneal swelling much of which occurs when corneal endothelial cells are compromised.

The normal corneal stromal swelling pressure is 60 mm Hg. If the endothelial barrier is disrupted the intraocular pressure of 15 mm Hg is unopposed and thereby aqueous seeps into corneal stroma leading to stromal swelling.

The optical function is contributed by both the anterior and the posterior surfaces. The refractive power at the air-tear interface is about +44 dioptres, +5 dioptres at the tear-cornea interface and -6 dioptres at cornea-aqueous interface.

REGENERATION/ HEALING MECHANISM IN CORNEA:

A.Epithelial healing:

The superficial epithelial cells are constantly shed into the tear pool and the epithelium renews itself within approximately 5 to 7 days. Unipotent stem cells which are located in the basal epithelium of corneoscleral limbus is thought to be responsible for the maintenance of the corneal epithelium. Wiley and associates used immunohistochemical staining for antibodies to keratins [7]and found that regional heterogeneity exists and that the limbus and superior corneal periphery contain the maximum number of stem cells producing replacement epithelial cells.

b. stroma: the response to any injury involves the following phases:

-removal of damaged tissue: the primary response will be the keratocytes death adjacent to the wound site which can last long upto one week followed by an inflammatory response

- proliferative and migratory phase: the nucleoli increase in number and size. Remodeling of the cytoskeleton takes place and the keratocytes get transformed into fibroblasts.

-phase of repair and replacement: the fibroblasts proliferate after the repair cells migrate into the acellular zone. Contraction of the myofibroblasts is associated with scar formation.

-termination of the wound healing process: within three to six Days post injury cell numbers return to normal.

-endothelium: cell migration and the enlargement rather than proliferation contributes mainly to the corneal endothelial wound healing. Thus the injury results in both pleomorphism and polymegathism(i.e cells with abnormal shape and size.)

DRUG PERMEABILITY ACROSS THE CORNEA: Topical route in the form of eye drops is commonly used as it has the advantages of : the drug effect localized to the area where it is needed , avoids hepatic metabolism, is easy and convenient, minimal systemic absorption. The following factors affect the drug penetration through the layers of cornea [8]:

1.water and lipid solubility of the drug:

Hydrophilic compounds have more resistance to absorption from the corneal epithelium while the lipophilic drugs encounter resistance in infiltrating the stromal layers.Lipophilic compounds are preferred more for corneal absorption as epithelium is the first and the main barrier for the drug absorption into the eye.

2.concentration, molecular size and weight of the drug

3.ionic form of the drug: ionized forms can pass through the stroma while non ionized forms can pass through the epithelium.

4. Ph of the solution

5. tonicity of the solution

6.surface active agents

NORMAL DEFENSE MECHANISM AGAINST CORNEAL INFECTIONS:

Cornea acts as a major defense mechanism against microbial infections. The corneal epithelium acts as a mechanical barrier while the pre-corneal tear film's components act as a major biologic protective system.the anatomical barriers to corneal infection the bony orbital rim, eyelids, intact conjunctival and corneal epithelial surface [3]. The mechanical barriers include blinking mechanism,tear film and punctual drainage system.

CORNEAL ULCER: corneal inflammation associated with significant loss of epithelium is called corneal ulcer. Microbial keratitis is the commonest causative factor of corneal ulceration which is important because of its sight threatening nature.

AETIOPATHOGENETIC CLASSIFICATION OF MICROBIAL KERATITIS:

a.bacterial- gram positive

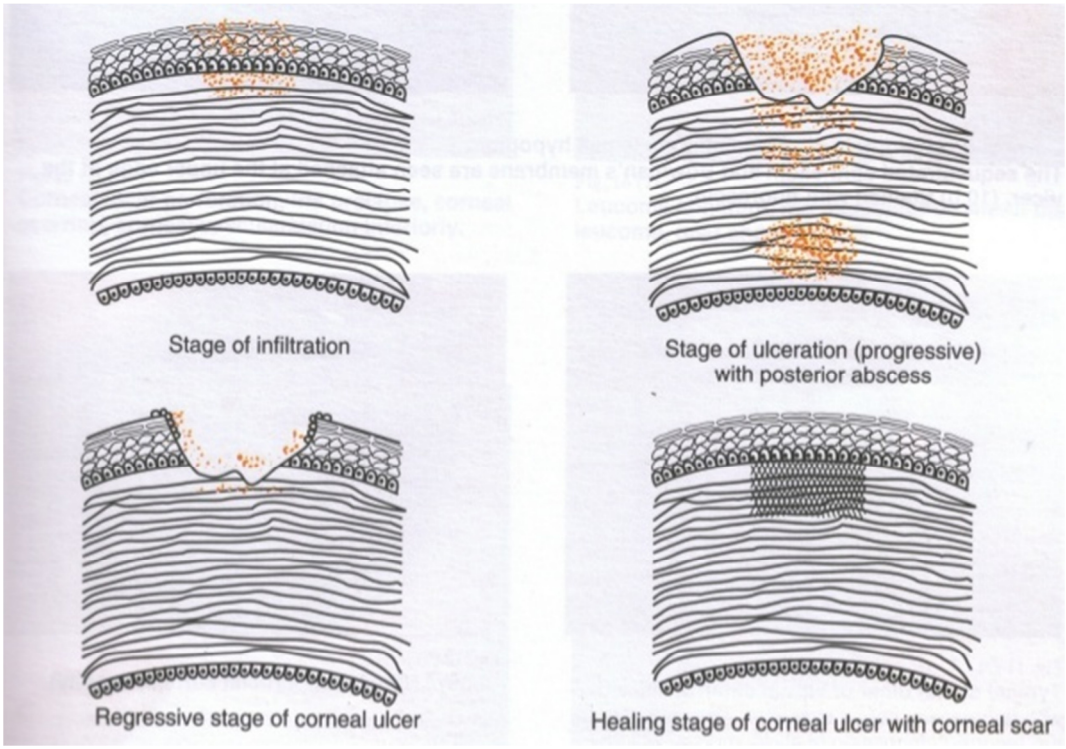
gram negative

b.fungi- filamentous, yeast

c. parasitic-protozoa

d.viral keratitis

STAGES OF CORNEAL ULCER:



1.**PROGRESSIVE STAGE:**

Adhesion of microbes to the corneal epithelium and release of toxins and enzymes causing tissue destruction.

Saucer shaped ulcer associated with grey zone of infiltration

Projecting walls of the ulcer

2.REGRESSIVE STAGE:

Host's defense mechanisms and the antimicrobial treatment is responsible for this stage

Symptomatic improvement noted along with decrease in signs

Margins and floor of the ulcer become smooth with line of demarcation noted.

Superficial vascularisation too noted.

3.HEALING STAGE:

Epithelisation begins and scar tissue is formed as keratocytes are converted to fibroblasts.

Vascularisation at the ulcer site results in influx of antibodies and fibroblasts thereby promoting healing.

After complete healing vessels regress and become ghost vessels.

Kenyon et al: inflammatory mechanisms in corneal ulceration reported different stages of ulcer. [4]

FUNGAL INFECTIONS OF EYE:

Fungi can infect almost any structure of the eye including cornea, conjunctiva, uveal tract, lens, ciliary body and vitreous. Predisposing risk factors include trauma, contact lenses, topical steroids and immunocompromised state.Two main factors that has lead to increase in the incidence of fungal corneal infections include an altered symbiotic relationship between the bacteria and fungi due to antibiotic usage, and due to decreased resistance of tissues due to topical steroid usage which makes the normally saprophytic fungi to become facultative pathogens.

FUNGI are commonly opportunistic invaders in compromised corneas as well as after trauma with plant or vegetable matter. Fungi have characteristic properties like their ability to survive at low redox potential of tissue, grow at body temperature and neutralize the host defense mechanisms.

In 1857, Virchow coined the term mycosis. It was then the first description of fungal keratitis being made. In 1879, Leber first reported Aspergillus as the etiological agent for a case of corneal ulcer with hypopyon.In 1913,cavara reported Mucor as causing keratitis.Romano yalour et al described candida albicans causing

typical clinical picture of corneal ulcer with hypopyon.In 1951, s.fazakas first found Cryptococcus to cause deep and extensive corneal involvement leaving little opacity

EPIDEMIOLOGY

INCIDENCE and GEOGRAPHIC DISTRIBUTION:It is greater in developing countries.its an important cause of morbidity and blindness in tropical countries and is attributed to the fact that the main working population in these countries are engaged in the agricultural industry where they are exposed to injury with vegetable matter.

Srinivasan et al (2004) reported fungal keratitis as enormous public health problem in south india with fungi accounting for 44% of corneal ulcers among which fusarium accounted for 47% and aspergillus 16%. [37]

Review of Indian literature reveals that the common fungal species causing fungal keratitis are aspergillus and fusarium .[5]

Fungal keratitis is more common in rural areas and warm climates. Of late fungal keratitis is increased in incidence due to the following factors:

a) Indiscriminate use of antibiotic-steroids and immunosuppressants

b) Greater incidence of clinical features recognition

c) Improved lab techniques.

d) Better reporting

SEX DISTRIBUTION: it tends to occur more commonly in males than females.[1]

Thomas PA: Fungal infections of the cornea[2]

AGE DISTRIBUTION: it is common in the age group 21-50 years.[2]

SEASONAL VARIATION:a higher incidence of these infections occur during early winter,And monsoons because of the high humidity and during the harvest season because of greater incidence of vegetative injuries during that period.

Hagan et al: causes of suppurative keratitis in Ghana- studied that the incidence is common in tropical climates

Coster DJ et al in 1981 demonstrated that the incidence of fungal keratitis remains very low in temperate climates.

RISK FACTORS IN THE DEVELOPMENT OF FUNGAL KERATITIS:

Wilson et al and Choudhry et al studied different agents of oculomycosis[7]

Ocular factors include

- Trauma :trauma is the most common risk factor for these infections. Keratomycosis in children is usually associated with trauma with vegetative matter. higher incidence of filamenous fungi being isolated after trauma with vegetable matter as these are implanted directly by the injuring material. These usually infect normal eyes of healthy young individuals who have mild corneal trauma with vegetable matter. this is frequently noticed in farmers and outdoor labourers [6]
- ocular surface problems including dry eye, bullous keratopathy, exposure keratitis,
- contact lens wear: 3-12% of infections are associated with contact lenses. Yeasts are commonly associated with bandage contact lenses while filamentous fungi commonly with aphakic or cosmetic contact lenses. Predisposing factors to contact lens related infection include improper lens care and topical steroids in patients with chronic epithelial defect among therapeutic lens wearers. [11]
- Drug abuse mainly corticosteroids and anaesthetics[8] .corticosteroids suppress ocular immune mechanisms by inhibiting chemotaxis and ingestion by phagocytes. They also decrease the production of phagocytes and block degranulation too. In 1953, Thygeson and his colleagues were the first to suggest the role of corticosteroid usage in the

development of fungal infections.In 1959, Anderson et al proposed the role of suppression of bacterial growth by antibiotics in the proliferation of fungi. In 1956, ley ; in 1964 van winkle et al : fungal corneal reaction is less severe when penicillin is used therapeutically in cases of mixed corneal ulcers where as ulceration tends to be more severe when antibiotics are added in absence of secondary infection .

- post any ocular surgery including cataract surgery, keratoplasty and refractive surgery. Predisposing factors are loose sutures, contact lens wear, chronic antibiotic- steroid usage, graft failure and persistent epithelial defects for the development of fungal keratitis in patients post keratoplasty.[14]

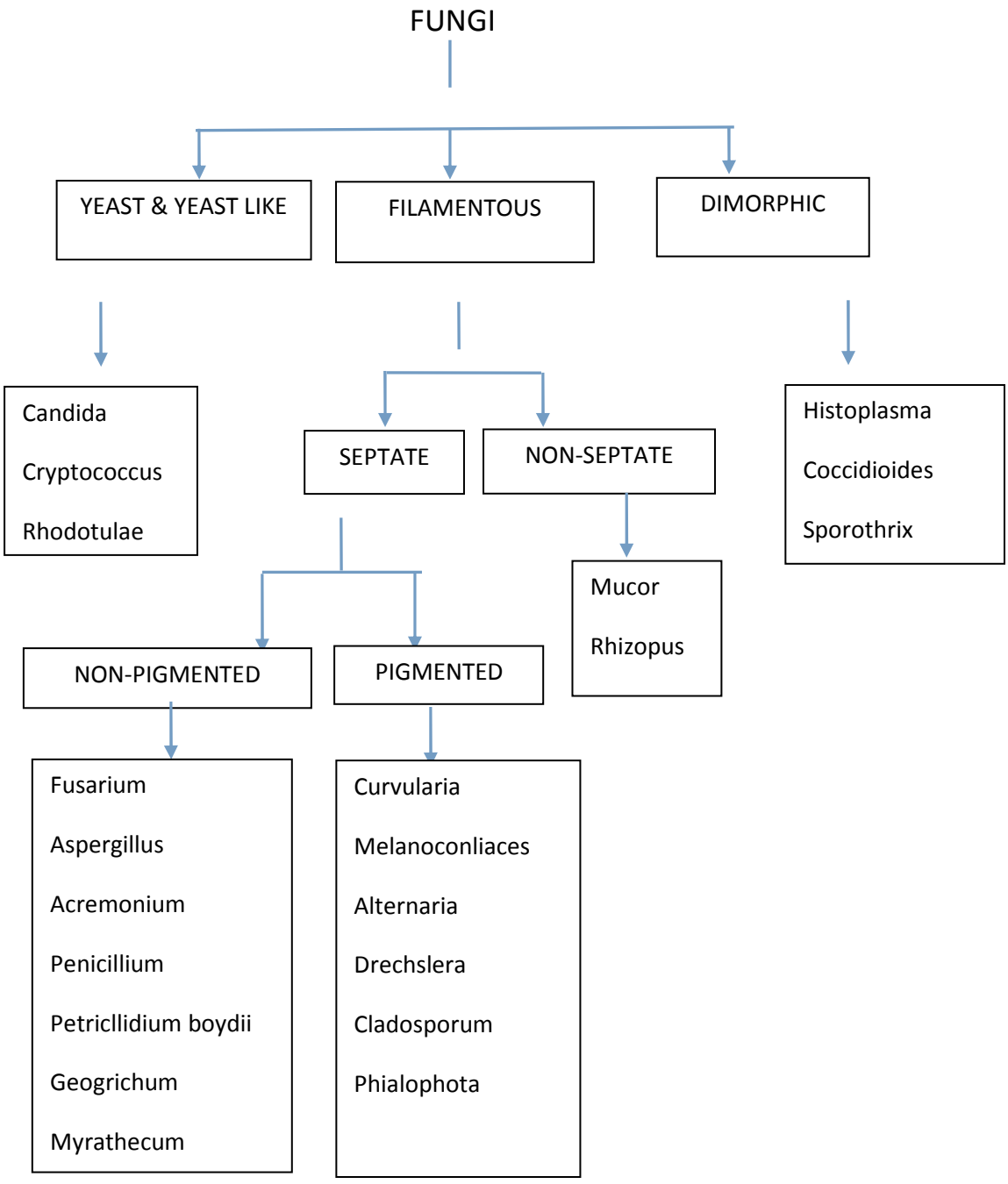
Following refractive surgery fungal keratitis can occur either in the early period due to direct surgical contamination or in the late period due to trauma.

- The systemic factors include immunocompromised state, diabetes mellitus, patients with chronically debilitated diseases. HIV positive patients are more likely to develop bilateral fungal keratitis compared to non –HIV positive patients. Candida and yeasts are more frequently opportunistic than filamentous fungi.

GENERAL MYCOLOGY:

Fungi are complex eukaryotic organisms having a nucleus, membrane bound granules, a rigid cell wall containing polysaccharides, chitin and ergosterol. fungal cells are larger than bacteria. Fungal virulence factors include capsule production to inhibit phagocytosis , cytokines production to depress immune system, cell wall polysaccharides that activate complement cascade inciting an inflammatory reaction or secretion of cytokines and mycotoxins that directly damage the host tissues.

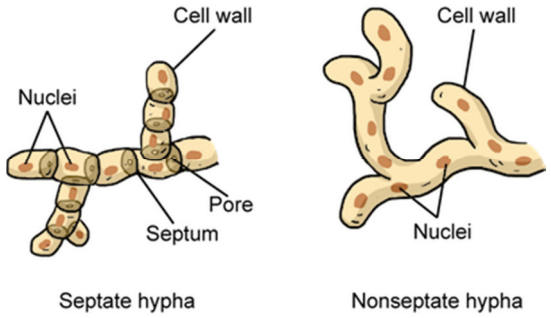
MORPHOLOGICAL CLASSIFICATION OF FUNGI



YEASTS: these are unicellular organisms characterized by a round blastoconidium. They reproduce asexually by budding. In budding the nucleus will undergo mitosis and a daughter cell pinches off to produce a new cell containing the new nucleus.These are characterized by pseudohyphae which form under reduced oxygen tension in the tissue .This pseudohyphal phase constitutes the most virulent phase. [7]

YEASTS LIKE FUNGI: these organisms grow partly as yeast and partly as elongated cells resembling hyphae which forms pseudomycelium.

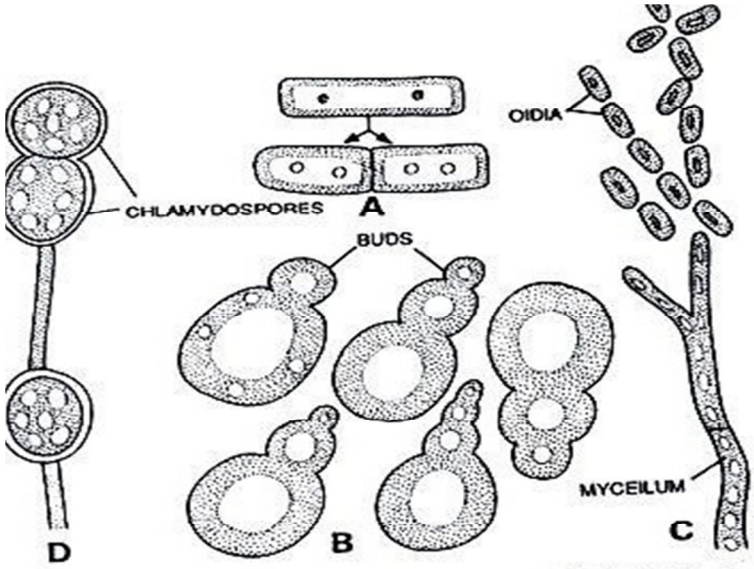
FILAMENTOUS FUNGI/MOULDS: these are multicellular organisms which possess hyphae and grow by either branching or apical extension. Hyphae are rigid tubes containing cytoplasm that moves towards the growing tip continuously to supply it with materials for growth.They reproduce either asexually by spore formation or sexually. They can be classified as septate or non septate based on the presence or absence of cross walls in the hyphae.



The septae have pores through which cytoplasm and nuclei pass to reach the growing tip. Septate hyphae possessing fungi cause most cases of fungal keratitis. Predominantly etiological agents are non pigmented fungi like fusarium species, aspergillus, penicillium and acremonium. Pigmented fungi include alternaria, bipolaris, curvularia, phialophora and lasiodiplodia.

DIMORPHIC FUNGI: these can occur as yeast or as filaments based on the growing conditions. They occur as yeasts at 37 degees C and at 22 degreesC appear as moulds. These rarely cause keratitis. Blastomyces, histoplasma, coccidioides and sporothrix come under this category.

Fungi reproduce generally by forming spores which may be asexual (mitosis only) or sexual as a result of fusion of two cells followed by meiosis. These asexual spores of medically important fungi are called conidia.



sexual reproduction. A, transverse cell division (fission); B, budding in yeast cell; C, hypha into oidia or arthrospores in *Collybia conigena*; D, chlamydospore formation in *Fusarium*.

MAJOR CLASSES OF FUNGI BASED ON SEXUAL SPORES:

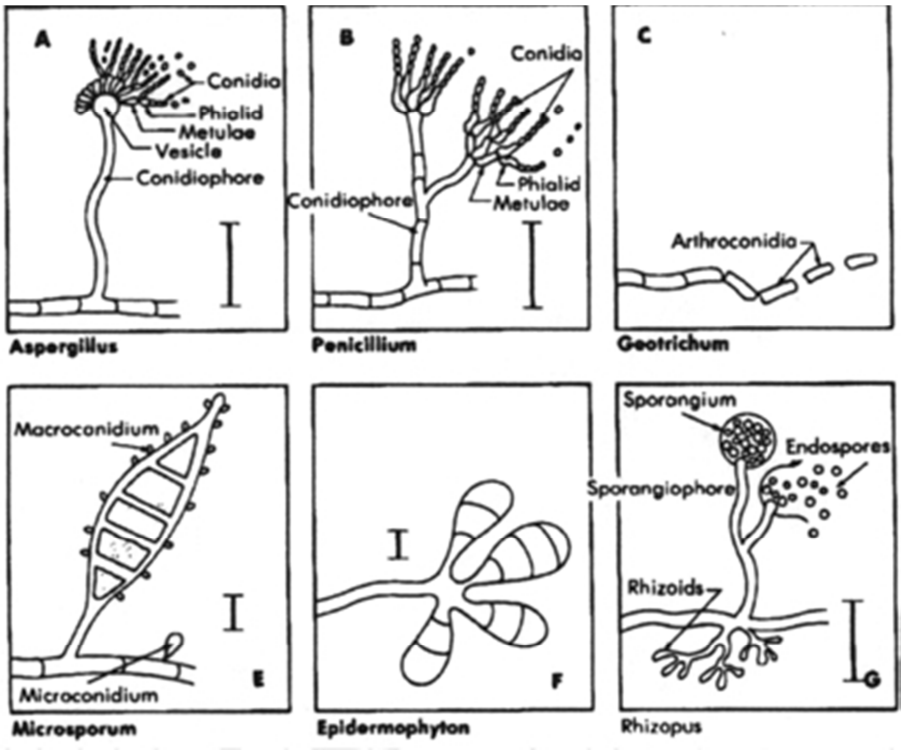
1.ZYGOMYCETES: These are septate filamentous organisms which are rare human corneal pathogens.

2.ASCOMYCETES: Genera Aspergillus and Penicillium come under this class of septate fungi which contain spores in their sacs or asci.

3.BASIDIOMYCETES: These septate fungi have club shaped structures called BASIDIA which has their sexual spores. This class includes mushrooms and plant rusts.

4.DEUTEROMYCETES OR FUNGI IMPERFECTI: This class lacks sexual spores and includes most human corneal pathogens. These are

again further divided into Moniliaceae and Dematiaceae based on the colour of hyphae. White or light coloured hyphae containing moulds are called hyaline fungi and come under the Moniliaceae group. Dematiaceae group contains moulds with brown or black pigmented walls. this pigmentation is because of the melanin contained in the fungal hyphal walls.



MORPHOLOGY OF FUNGI:

These are saprophytic organisms as they are plant like but lack chlorophyll .fungal cell wall is composed of polysaccharides and lipids. 80-90% of fungal cell wall is made up of polysaccharides which is usually cellulose or chitin. Unlike the animal cell wall containing cholesterol ergosterol is the sterol here.

FUNGI MORPHOLOGY

YEAST AND YEAST LIKE(UNICELLULAR FUNGI THAT REPRODUCE BY BUDDING) :

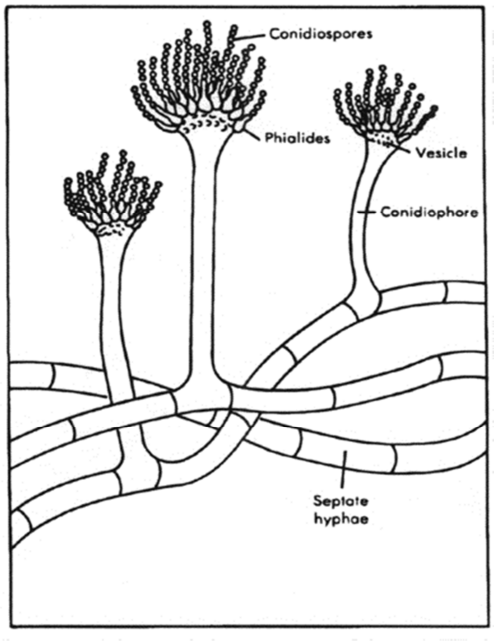
Macroscopic morphology: these form non mucoid creamy textured smooth colonies

Microscopic morphology: these are round to oval shaped budding cells which can produce germ tubes which are parallel sided tubes and are formed at right angles to the parent cell.

ASPERGILLUS SPECIES:

Aspergillus is a large genus with many species out of which A.fumigatus and A.niger are particularly important.Their hyphae have dichotomous branching which is a diagnostic feature. The conidiospores have swollen terminal vesicles surrounded by flask shaped sterigmata each of which produce long chains of

coccoid conidia radiating out from the vesicle. Three of aspergillus species are common corneal pathogens.



Aspergillus flavus:

Macroscopic morphology: colonies are flat,granular often with radial grooves yellow to green in colour

Microscopic morphology: conidial heads are mostly 300-400 micron in diameter and typically radiate. Biseriate heads but some with phialides borne directly on the vesicle.conidiophores are hyaline and roughened.

Aspergillus fumigatus:

Macroscopic morphology: colonies show typical blue green surface pigmentation with a suede like surface with dense felt of conidiophores.

Microscopic morphology:

Conidial heads are typically columnar, uniseriate. Conidiophores are short, smooth walled with conical shaped terminal vesicles . These have one row of phialides on upper two thirds of the vesicle. Conidia are green, globose and rough walled and are produced in basipetal succession forming long chains.

Aspergillus niger:

Macroscopic morphology:colonies are white or yellow in colour covered by a dense layer of dark brown to black conidial heads.

Microscopic morphology: conidial heads are globose large dark brown with smooth walled hyaline conidiophores with biseriate conidial heads .the conidia are dark brown to black, globose and rough walled.

2.FUSARIUM:

Macroscopic morphology: they produce wooly to cottony , flat , spreading colonies.

Microscopic morphology:

They are characterized by the large banana shaped or sickle shaped conidia which are produced on short lateral hyphae-conidiospores.

3.CANDIDA:

Macroscopic morphology: these colonies are convex non mucoid cream coloured colonies

Microscopic morphology: They have round oval shaped budding cells. True germ tubes are produced which are parallel sided tubes formed at right angles to the parent cell when incubated in serum for 2-3 hours at 37 degrees.

4.DEMATIACEOUS FUNGI:these are generally darkly pigmented because of the melanin present in their hyphal walls.this characteristic dark colour is generally noticed on potato dextrose agar and often appear salmon coloured on saboraud's dextrose agar.

In Garg and colleagues study[16], only 27% of the dematiaceous fungi caused corneal ulcers appeared to be darkly pigmented macroscopically. they studied 88 cases of keratomycosis caused by dematiaceous fungi in india among which 50% could not be further identified as they did not sporulate and of the remaining half curvularia was the most common species (23%) followed by exserohilum and bipolaris(15% combined)

-CURVULARIA: Mostly they are facultative pathogens with curvularia lunata being the most commonly encountered species. It has now emerged as an opportunistic pathogen infecting immunocompromised patients.

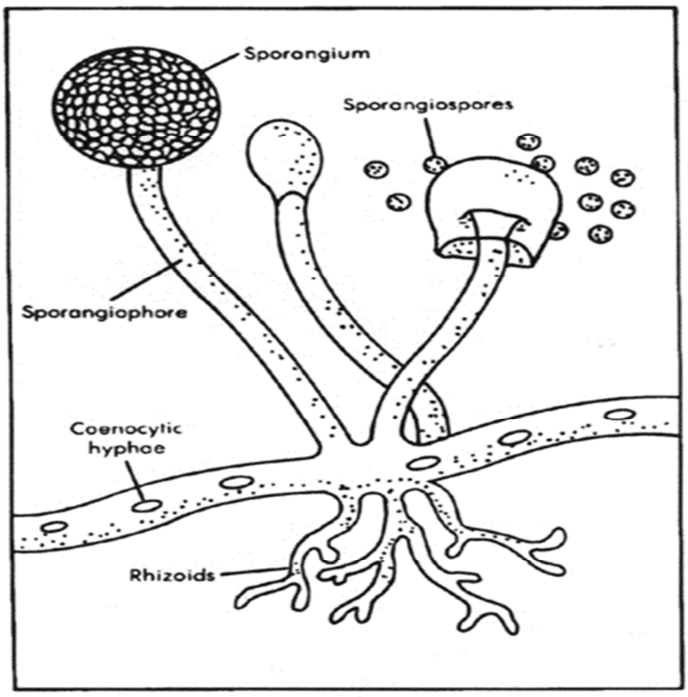
-ALTERNARIA: Most common fungus isolated in this group. It has also emerged as an opportunistic pathogen.

-AUREOBASIDIUM PULLULANS: It comes under phaeohyphomycoses group. These usually cause centrally located ulcers with multiple round ball like infiltrates around the ulcer.

RHIZOPUS:

Mcroscopic morphology: cotton candy like texture of these rapidly growing colonies.front surface of the colony is white initially which turns grey to yellow brown later while the reverse surface is pale white.

Microscopic morphology: these have broad hyphae which may be either non septate or sparsely septate.they have brown colour sporangiophores on the tip of which round sporangia are located with flattened bases. At the junction of stolons and sporangiophores rhizoids are located.



PATHOGENESIS OF FUNGAL CORNEAL ULCER:

Ocular infection occurs as a result of breach in the normal healthy interaction between three important factors- the host, pathogen and the environment.

The pathogenicity of the fungi is dependent on the characteristics of the invading organism and the status of the normal defenses of the host. The inflammatory reaction results from:

- Damage caused by fungal toxins and proteolytic enzymes.
- Fungal invasion and growth causing direct physical damage.
- Damage caused by infiltrating leucocytes.
- Soluble fungal antigens

Host leukocytes infiltration with ring abscesses composed of plasma cells, PMN leukocytes and eosinophils are characteristic of fungal corneal ulcer.(zimmermann, naumann G, Green)

Neutrophils destroy fungal hyphae and damage the surrounding tissue via exaggerated phagocytosis thereby releasing oxygen metabolites and lysosomal enzymes.(Diamond et al).

Few studies have investigated the role of fungal enzymes and toxins as contributing factors in pathogenesis. Few strains of aspergillus produce aflatoxins and ochratoxins whose role in corneal infection is not known.other toxins include trichothene toxins produced by fusarium and acremonium species which elicit inflammation at low doses and destroy many cell types at higher concentrations. Other toxin is the gliotoxin produced by penicillium, aspergillus and gliocladium species which are epipolythiodioxopiperazines having antibacterial, antiviral, antitumor and anti-phagocytic properties. Canditoxin is produced by canida albicans species.it also produces proteolytic enzymes- aspartyl acid protease, neutral and carboxyl proteases and phospholipaes.

The destructive potential varies from the rapidly destructive Fusarium to lesser virulent acremonium species. Fungi can penetrate the stroma, attack descemets membrane and enter the anterior chamber unlike the bacteria. Multiple virulence

factors act on the host like the cell wall polysaccharides that provokes inflammatory reaction by activating the complement cascade.

HOST IMMUNITY:

Host's cell mediated immunity play an important role in protection against fungal infection. Neutrophils contain myeloperoxidase and hydrogen peroxide which attributes to its fungicidal property.

CLINICAL EXAMINATION OF A CASE OF CORNEAL ULCER:

- general examination
- visual acuity recording
- eyelids for the presence of any abnormality like trichiasis, ectropion, entropion, lagophthalmos
- nasolacrimal duct patency
- slit lamp examination- to look for the size and depth of the ulcer, anterior chamber reaction, associated characteristic findings.
- intraocular pressure.
- Posterior segment evaluation to be done in cases of suspected endophthalmitis.

CLINICAL PRESENTATION OF FUNGAL KERATITIS:

In 1965, Kaufman and Wood described the salient features of mycotic keratitis.

SYMPTOMS:

- Gradually increasing pain
- Defective vision: central corneal ulcers particularly caused by fusarium are invariably associated with significant loss of visual acuity.
- Foreign body sensation

SIGNS:

- Circumcorneal injection
- Early features include granular infiltration upto the anterior stroma with minimal cellular reaction.
- Dry looking ulcer with feathery margins with rough texture and dirty grey white colour.
- Elevated edges
- Satellite lesions or multifocal suppurative microabscesses.
- Anterior chamber inflammation, Fixed immobile hypopyon and endothelial plaque usually parallels the density and size of the lesion. Hypopyon usually results from sterile reaction to fungus and its toxins. But however the fungi can invade the anterior chamber through intact descemet's membrane and

forms a fixed hypopyon.thus the exudation in the anterior chamber is not sterile unlike the bacterial ulcers.

- A white ring noted representing the interactive junction of fungal antigen and host antibody or toxic fungal diffusate.
- In dematiaceous fungal keratitis pigmentation in the ulcer bed is noted occasionally.
- The entire cornea becomes homogenously white in advanced disease.
- endothelial plaque: It is composed of fibrin and leukocytes. It is located under the stromal lesion and can be present even when hypopyon is not present.

SPECIFIC CHARACTERISTIC FEATURES OF KERATITIS

1.BY FILAMENTOUS FUNGI:

- Most often followed by a history of trauma with vegetable matter.
- Can involve any part of the cornea
- Ulcer characterized by elevated grayish white infiltrate with hyphate margins and surrounding satellite lesions which are discrete stromal abscesses separated by clear cornea.

- Irregular elevated feathery margins and endothelial plaques composed of inflammatory cells seen.
- Hypopyon commonly associated.
- F.solani keratitis often causes stromal ulceration and necrosis leading to perforation and endophthalmitis. Acremonium species causes dense suppuration.

2.BY NON FILAMENTOUS FUNGI:

- Most commonly seen in eyes with preexisting surface abnormalities, yeast keratitis commonly occurs in association with systemic diseases such as immunoglobulin A deficiency, cell mediated immunodeficiency.
- Ulcer site usually at the junction of upper 2/3rd and lower 1/3rd.
- Usually no feathery margins, no hyphate edges, no satellite lesions.
- Small oval ulceration with sharply demarcated dense stromal suppuration that lacks the typical delicate features of filamentous keratitis.

SEVERITY GRADE OF MICROBIAL KERATITIS:

Jones gave the following criteria for the severity grading of ulcer based on the signs:

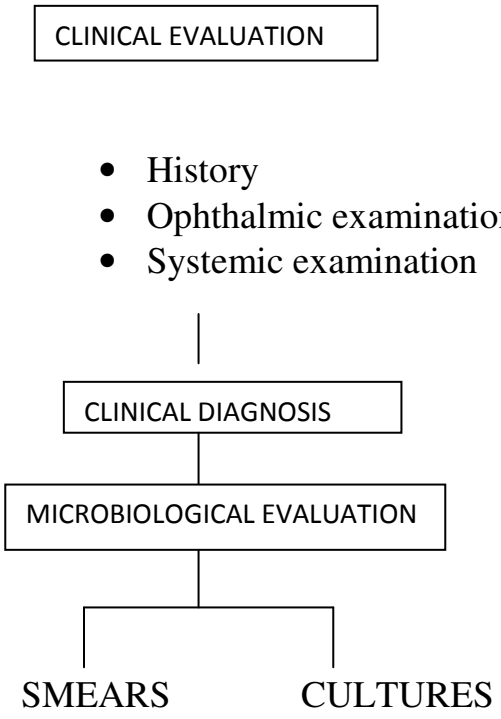
FEATURE	NON-SEVERE	SEVERE
Rate of progression	Slow, moderate	Rapid
Area of suppuration	< 6 mm diameter	>6 mm diameter
Depth	Superficial 2/3 rd	Inner 1/3 rd
Depth of ulceration	Superficial 1/3	Inner 1/3
Perforation	Unlikely	Present, imminent
Scleral suppuration	Absent	Present

Depending on the severity ulcers may be graded as follows:

Factor	GRADE 1	GRADE 2	GRADE 3
LOCATION	Non axial	Central/peripheral	Central/peripheral
AREA	2 mm	2-6 mm	>6 mm
DEPTH	Superficial $1/3^{rd}$	Superficial $1/3^{rd}$-$2/3^{rd}$	Extending to inner $1/3^{rd}$
anterior segment inflammation	None/mild	Moderate to severe fibrinous infiltrates	Hypopyon

DIAGNOSIS:

Flowchart for diagnosis and isolation of causative organism in infectious keratitis:



Laboratory techniques: there are some guidelines for proper procurement and examination of specimens.:

-gram and giemsa stains are absorbed by the protoplasm of filamentous fungi but do not stain the septa of fungal hyphae or the cell wall.

-oil immersion is not necessary and high magnification alone is sufficient to detect fungal organisms.

Jones et al: key to positive isolation of the causal organism in fungal infections is careful attention to diagnostic techniques and repeated attempts to isolate the microorganism in culture.

The elements are rarely present in corneal smears that allow classification:

Aspergillus- small hyphae with distinct cross septa and branching at 45 degrees.

Nocardia- gram positive, acid fast partially with delicate branching filaments.

Candida- budding characteristically noted in yeast forms

Yeasts- stain dark blue typically.

Corneal scraping: Smears are taken from the ulcer margins and bed, lid margins and conjunctival sac under topical anaesthesia using bard parker 15 blade or with platinum loop after obtaining consent from the patient. Care is to be taken not to perforate the thinned areas and the direction should be always towards one side only.These smears are prepared on pre cleaned glass slides and fixed with 5% methanol. These slides are processed for the following stains after keeping one slide in reserve[16]

i. Grams stain- it stains the fungal protoplasm selectively. Most fungi are gram positive. Filamentous fungi have varying staining response while

yeasts are gram positive. This method is also useful to identify any coexixting bacterial infection.

ii. PAS stain- PAS reagent hydrolyses and oxidizes cell wall polysaccharides and stain the hyphae as bright red.

iii. Giemsa stain- it is also a selective stain like grams stain. It imparts purplish blue colour to the corneal fungal pathogen. It highlights the cytoplasm and septum

iv. Grocott gomori methenamine silver stain: it is the most selective method for identifying the fungal elements but is a costlier and cumbersome technique. Black staining is noted as reduction of silver occurs by the oxidized carbohydrate components of the fungal cell wall.[15]

v. Calcofluor white and acridine orange are other stains which are easy to perform giving rapid diagnosis but the disadvantage is that it requires fluorescent microscope.

KOH MOUNT: 10%KOH wet mount can be used to detect fungi. KOH retains only the fungal filaments by dissolving the cells and debris. [19]It has got low sensitivity as only 1/3 rd of the fungal keratitis are positive by this method. In addition to fungal filaments, acanthamoeba cysts and nocardia filaments can also be visualized.

- When examined with UV light candida and aspergillus exhibit autofluorescence.
- Lectins when conjugated with fluorescein can be seen under the fluorescence microscope. These bind to the cell walls of yeast, filamentous fungi and atypical mycobacteria.; cysts and trophozoites of acanthameba

CULTURE:

The isolate is transferred directly to the culure plate by making a row of C-shaped marks reversing the edge of the spatula with each C, so that the entire material on spatula is transferred to the plate. This process is repeated several times until several rows of C-shaped streaks have been made on each solid plate. Although most fungal isolates will be recovered within 48 hrs of inoculation, at least 25% may require incubation as long as 3 weeks before any growth is recognized.

CULTURE MEDIA EMPLOYED FOR FUNGAL RECOVERY:

- BLOOD AGAR: this media supports the growth of most fungi as well as bacteria. Two plates are inoculated, one at body temperature and the other one kept at room temperature for fungal growth.

- SABOURAUD'S DEXTROSE AGAR MEDIUM: sabouraud originally described the SDA agar formulation which contains 4% glucose. It contains dextrose, peptone, agar along with 50 mg of gentamycin to inhibit bacterial growth but it should not contain cycloheximide as it might inhibit some saprophytic fungi.[14] It is an universal readily available nonselective medium for the purpose of primary isolation of fungi.to improve nutritional characteristics yeast extract is added. Fungi are transferred to the sporulating medium after the primary growth. Sabouraud agar plates are generally preferred over the slants because of:

  -colony growth can be observed

  -ease of inoculation

  -dilution of inhibitory substances

  - transfer to secondary media

  The specimens are incubated at room temperature in sabourauds agar and the agar is thicker than usual medium.

- LIQUID BRAIN HEART INFUSION RECOVERY: this liquid media offers good recovery of fungi and can be used as an adjunct to solid media. This broth contains beef heart infusion and calf brain with dextrose and protease, neomycin and gentamycin too are added to enhance fungal groth and to control bacterial contamination respectively.

- LACTOPHENOL COTTON BLUE MOUNTING MEDIA: It contains phenol, lactic acid, glycerol, cotton blue and distilled water. It is used to identify and evaluate different fungal colony characteristics.

ORGANISM	EARLY	LATE
Aspergillus fumigates	White	Velvety green
Aspergillus niger	White	Turns black as sporulation begins
Fusarium	White	Acquires buff colour as colony matures
Acremonium	Compact	Develops wooly appearance

- OTHER SPECIAL MEDIA:

  These include potato dextrose agar, cornmeal agar and czapek dox agar.

  JONES CRITERIA FOR DIAGNOSIS FROM CULTURE:

  Clinical signs of infection plus bacterial isolation (10 or more colonies)on one solid medium and one additional medium or isolation of fungi (any detectable growth) on any two media or one medium in the presence of smear being positive.

The colour, growth rate, surface texture, microscopic appearance and pigmentation on the reverse side are the initial identifying features microscopically.

CORNEAL BIOPSY: this gives a better yield than corneal smears for recovering fungi. Alexandrakis et al demonstrated from the corneal biopsy specimens increased recovery rate in cases of recalcitrant culture negative cases.

ANTERIOR CHAMBER TAP can be used to isolate fungal organisms that penetrate intact descemet's membrane. The hypopyon and endothelial plaque can be aspirated under aseptic conditions and submitted for laboratory examination. In suspected progressive fungal infection with repeated negative culture reports anterior chamber paracentesis can be indicated.

In POST LASIK cases sample collection requires special precaution. Here the surface scraping is not indicated for the fear of button holing of the flap. It should be performed after lifting of the flap and specimen is to be collected from both the undersurface and bed of the flap. In cases of flap necrosis amputation or excision of flap has to be done to decrease the load of infection.

- **NEWER HORIZONS IN DIAGNOSIS:**

-Polymerase chain reaction: it aids in diagnosing microbial keratitis and gives rapid results and also picks up cases even in partially treated cases [20].it is typically used in one of the following conditions:

a. When a patient does not respond appropriately to therapy
b. When a patient has signs and symptoms that are most likely an infection but a definite diagnosis is not arrived .
c. When a patient's history and clinical presentation does not coincide.
d. For diagnosis confirmation.

The ophthalmic samples for PCR are collected either from corneal scrapings , infected corneal button, anterior chamber paracentesis, or a vitreous tap.

-In vivo confocal microscopy for early detection of fungal keratitis.it provides observation of microorganisms in vivo without the use of any dyes or stains or tissue fixation. It precludes the need for invasive procedures like corneal biopsy in cases of deep seated infiltrations and delayed growth in culture. Yeasts grow in a plane perpendicular to the corneal stromal lamellae and appear as multiple round hyperreflective points while filamentous fungi traverse in a plane corresponding to the horizontal stromal lamellae and appear as multiple linear branching structures.

-Non specific fluorescent stains like uvitex 2B and Blankphor- rapidly detects fungal filaments in corneal scrapings

- **<u>OTHER FUNGAL IDENTIFICATION TESTS</u>**:
    i. Germ tube production
    ii. Biochemical test
    iii. Immunodiffusion
    iv. Latex agglutination tests
    v. Counter immunoelectrophoresis
    vi. Crossed electrophoresis
    vii. ELISA

HISTOPATHOLOGY: Two histopathological features considered to be suggestive of progressive pathogenicity include:orientation of fungal hyphal elements perpendicular to the normal corneal lamella and a tendency for the hyphal elements to penetrate intact descemet's membrane.

<u>DIFFERENTIAL DIAGNOSIS</u>:

- Bacterial ulcers especially pseudomonas . Yeast keratitis often resembles gram positive bacterial keratitis like staphylococcal aureus/ streptococcus pneumonia.
- Acanthamoeba keratitis
- Viral keratitis: filamentous fungal keratitis often mimics viral keratitis
- Atypical mycobacterial, nocardia ulcers
- Infectious crystalline keratopathy

TREATMENT:

The objective of therapy is to:

-to provide symptomatic relief for the patient.

-to reduce the inflammation.

-to eliminate the infective organism rapidly.

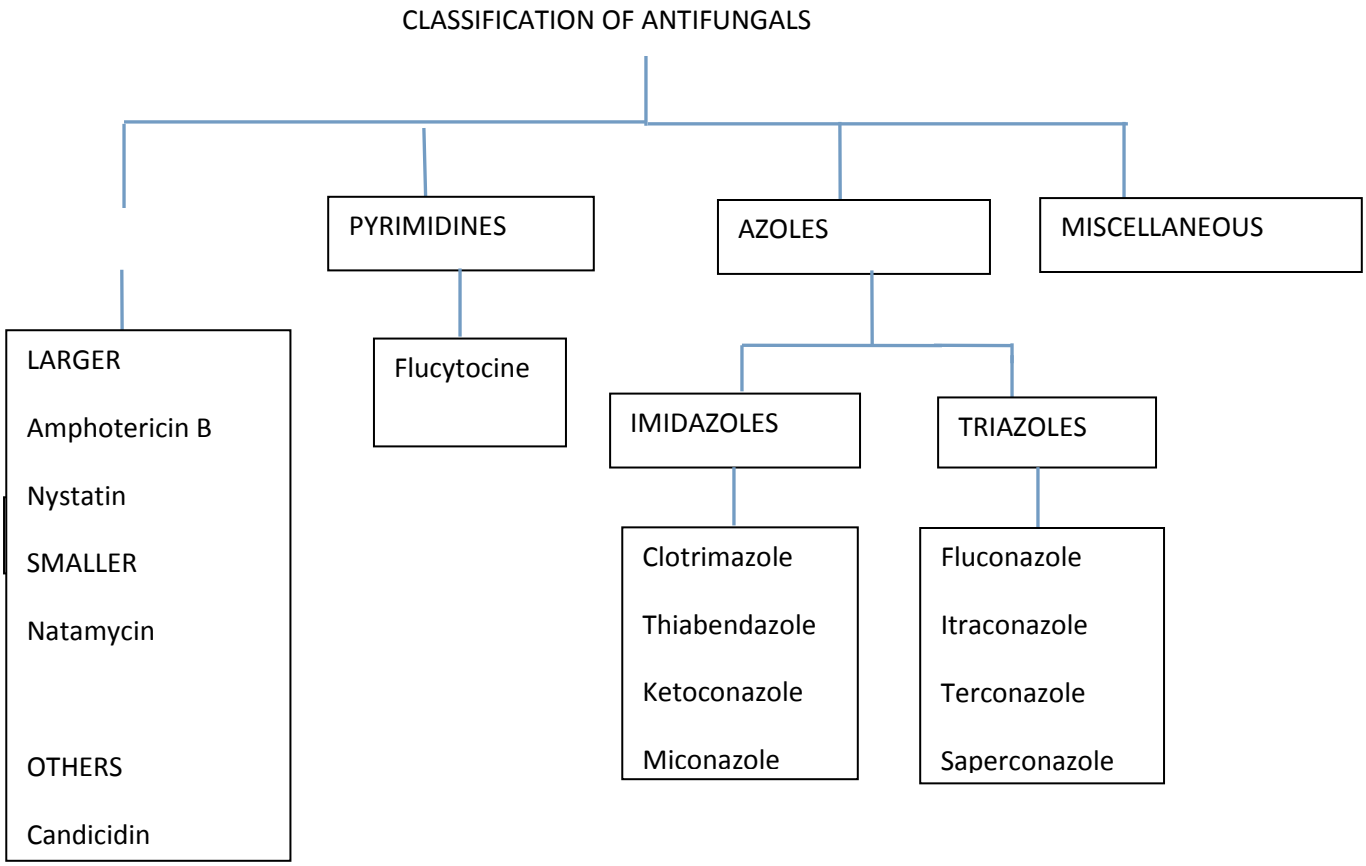
-to promote epithelial healing and provide structural damage to the cornea.

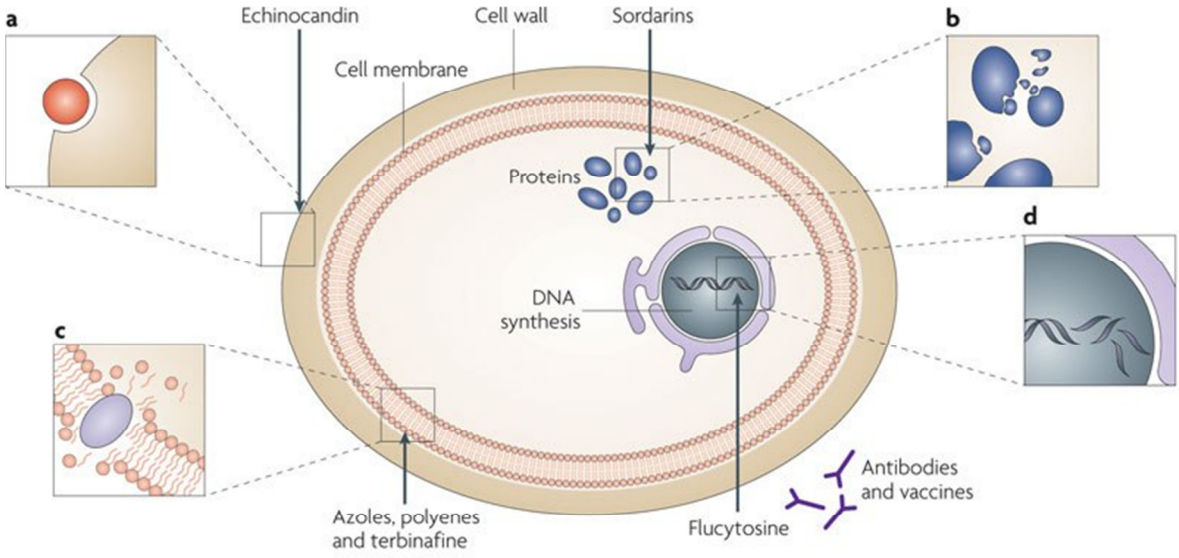
GENERAL CONSIDERATIONS IN TREATMENT:

Lalitha p et al, 2006- lack of effective antifungal agents responsible for inability to treat fungal keratitis despite diagnosis.[34]

Most of the antifungal agents act on ergosterol present in the fungal cell wall. The difference in the plasma membrane constituents between the fungi and the host cell has been capitalized on by the antifungal agents as they selectively damage the fungal cells while causing minimal damage to the host cells.

CLASSIFICATION OF ANTIFUNGAL AGENTS:





POLYENES: they can be classified as larger and smaller polyenes. Though both bind to sterols their subsequent effect on the cell membranes differ due to their relative size.the larger polyenes approximate the phospholipids present in the cell membrane and forms channels for the passage of potassium chloride and other ions leading to electrolyte imbalance and thereby cell death. This lethal effect can be blocked by the electrolyte control of the surrounding medium. Smaller polyenes accumulate on the membrane and form blisters instead of channels and disrupt the phospholipid film. This effect is not reversible by the electrolyte control.

NATAMYCIN: Smaller polyene antibiotic.

Mechanism of action: it binds to ergosterol in the cell membranes , increases cytoplasmic membrane permeability resulting in leakage of intracellular constituents. It is available as 5% suspension.it is the only FDA approved topical ophthalmic preparation.

SPECTRUM OF ACTIVITY:

It is routinely employed for keratitis caused by filamentous fungi and is usually more effective against superficial fungal infections that are not very severe as the drug has poor penetration into the deeper structures of the cornea.

Kalavathy et al did a prospective nonrandomized study comparing the efficacy of 5% natamycin with 1% itraconazole drops as monotherapy for fungal keratitis and concluded that natamycin is more effective than itraconazole in the treatment of Fusarium keratitis but is not that efficacious in the management of deep stromal infections.

1. Aspergillus
2. Fusarium
3. Curvularia
4. Acremonium

SIDE EFFECTS: it is well tolerated

1. Follicle formation

2. Conjunctival hyperemia
3. Persistent epithelial ulceration

Prajna et al in 2003 found no statistically significant difference between 5% natamycin and 2% econazole in a prospective comparative study among 112 cases of culture proved fungal keratitis.[30]

It is the preferred initial agent for treating filamentous keratits as it has a broader spectrum of activity, lacks toxicity and has been established as efficacious against various types of superficial corneal pathogens. Lack of absorption and tissue toxicity precludes its use particularly. It deposits on the cornea upon instillation.

AMPHOTERICIN B: This is a larger polyene antibiotic derived from strains streptomycin nooses.

MECHANISM OF ACTION: It is fungicidal at high concentration and fungi static at lower concentrations. It increases the permeability of cytoplasmic membranes by binding to ergosterol present in the cell membranes and permitting the leakage of intracellular constituents.

Spectrum of activity includes:

1. Candida

2. Aspergillus
3. Fusarium and other filamentous fungi
4. Cryptococcus

ROUTES EMPLOYED:

- TOPICAL- 0.15%. The parental formulation is diluted with distilled water without bacteriostatic agent to prepare the topical formulation.
- Subconjunctival: 1-2 mgs
- Intracameral route

Kaushik et al: three culture proven aspergillus flavus corneal ulver patients who did not respond to conventional 5% natamycin, 0.15% amphotericin B or oral itraconazole had complete resolution of the ulcer and hypopyon on receiving intracameral amphotericin B.

- Intravitreal routes
- The CLEAR-Collaborative exchange of antifungal research study was a retrospective study which reviewed the efficacy and renal safety in candida infected patients treated with lipid complex amphotericin B and concluded that lipid complex formulations of amphotericin B are effective and are at lesser risk of renal toxicity than conventional amphotericin B.

SIDE EFFECTS: nephrotoxic.

Higher doses produce conjunctival necrosis

1. Allergic reactions
2. Burning sensation
3. Follicular conjunctivitis
4. Punctuate keratitis
5. Delayed corneal wound healing
6. Uveitis
7. Subconjunctival injections as low dose as 0.1 mg can cause transient conjunctival nodules and also permanent yellow conjunctival discoloration

NYSTATIN:

It also belongs to polyene group of drugs which can be used as a topical suspension but it is no longer commonly used as it has only superficial activity in vivo

AZOLES: These antifungals are non selective and has the ability to inhibit many mammalian cytochrome P450 dependant enzymes. Therefore the medications metabolized through the P450 pathway have drug interactions with the azole group of antifungals.

Mode of action:

Fungistatic (inhibits ergosterol synthesis)at low concentration while fungicidal(direct damage to fungal cell wall) at high concentration. At concentrations used it shows only fungi static activity

KETOCONAZOLE:

It was the first successful broad spectrum oral azole antifungal. It has good activity against candida albicans but a moderate coverage of aspergillus species. It is available both orally and as topical 1% eye drops. Long term oral administration is associated with hepatotoxicity and can result in complications like gynaecomastia, alopecia or impotence

TRIAZOLES:

ECONAZOLE: it is a broad spectrum antifungal and 2% econazole applied topically has been found to be efficacious as 5% natamycin in treating fungal keratitis.

CLOTRIMAZOLE: it has a greater tendency to bind to fatty acids but is more toxic and cannot be administered systemically. It is available as a 1% topical preparation . Mselle treated twelve patients with proven fungal keratitis with clotrimazole but concluded that clotrimazole alone as a single agent will not be an ideal choice.

IMIDAZOLES:

ITRACONAZOLE: it is a broad spectrum synthetic triazole having less toxicity and good oral bioavailability. It can be used as 1% suspension . It is contraindicated in patients who have hypersensitivity to the drug or its components.

Agarwal et al did a randomized prospective study and concluded that topical itraconazole is effective in treating superficial less severe fungal ulcers but it may be less efficacious than natamycin in the treatment of Fusarium keratitis.

FLUCONAZOLE: it is a highly water soluble drug available as 0.3% topical preparation which acts primarily against Candida species. Oral and intravenous formulations are also available. It is very safe and has good corneal penetration . A major limiting factor associated with the use of fluconazole is that it has a narrow spectrum of activity.

VORICONAZOLE: It is a recently developed azole antifungal having broad spectrum of activity available as oral and intravenous formulation.

POSACONAZOLE: It is as active as voriconazole having a broad spectrum of activity with added activity against zygomycetes. It is a recently introduced drug and studies on its ocular penetration are lacking. It is a safe drug used both orally and as topical formulation.

ADVANTAGES OF TRIAZOLES OVER IMIDAZOLES:

- Lower toxicity
- Improved selectivity
- Increased stability
- Lower rate at self induced hepatic metabolism

PYRIMIDINES-FLUCYTOSINE: it interferes with protein synthesis by incorporating into the fungal RNA.It is available topically as 1% solution and systematically as 150 mg/kg/day. It is effective against Candida and has synergistic action when combined with Amphotericin B. It is not effective against fusarium or acremonium.capsules are dissolved in distilled water to obtain 1 percent topical solution. Oral flucytosine controls deep stromal suppuration or endophthalmitis as effective aqueous and CSF levels are obtained. Adverse effects include hepatic dysfunction, leucopenia, thrombocytopenia and gastrointestinal upset

ECHINOCANDINS:

These group of antifungal agents target the fungal cell wall by depleting glucan polymers in it by inhibiting glucan synthesis and cause a weak cell wall. These are selective in action but the oral bioavailability is poor in this group. Durand et al reported case report of two patients with aspergillus and fusarium endophthalmitis successfully treated with caspofungin in combination with voriconazole.

Matsumoto et al reported resolution of three candida ulcer patients with topical 0.1% micafungin who did not respond initially with topical polyenes and azoles.

TERBINAFINE:It is used as 0.25% eye drops and is effective as 5% natamycin against filamentous fungi especially in cases of smaller and shallower ulcers. It is active against few types of yeasts too. It is safe but requires longer duration of treatment.

In vitro minimum inhibitory concentrations of common antifungals

Antifungal agent	Aspergillus	Candida	Fusarium
Voriconazole	0.5	0.016	2
Amphotericin B	2	0.5	2
Itraconazole	1	0.256	>16
Fluconazole	>256	0.5	>256
Ketoconazole	4	0.032	>16

OTHER ANTIFUNGAL AGENTS:

Silver sulfadiazine

Povidone iodine 5%

0.2% chlorhexidine glucomate.

DURATION OF TREATMENT: The length of time required for topical treatment has not been firmly established. Different reviews have lead to the derivation of certain guidelines. Generally the time taken is longer than that for bacterial keratitis. It should be individualized based on clinical response. Jones et al reported an average duration of 30 days of treatment for fusarium keratitis with natamycin. Penetration of many topical antifungal agents is poor which can be aided by repeated scraping off the epithelium.

SYSTEMIC ANTIFUNGAL AGENTS :

These are employed in cases of

- Deep ulcers
- Very large ulcers
- Associated scleritis/limbitis
- Endophthalmitis

-INTRACAMERAL ROUTE:

This procedure when performed under strict aseptic conditions ensures adequate drug delivery into the anterior chamber.

-INTRASTROMAL ROUTE:

Amphotericin B intrastromal injections have been demonstrated to be useful in cases of recalcitrant mycotic keratitis.

Both intrastromal and intracameral routes are to be considered in cases with intraocular extension or with anterior chamber involvement.

SUBCONJUNCTIVAL: This route is usually not preferred because of the toxic effects of the drugs. Miconazole is least toxic and better tolerated for sunconjunctival route administration.

-**USE OF STEROIDS IN FUNGAL KERATITIS:**

This is controversial as it can worsen the infection. O'Day et al: reported that topical steroids when given alone worsened the disease and also adversely affected the efficacy of antifungal agents when given in combination. it is therefore recommended not to consider topical steroids until after at least two weeks of antifungal treatment and control of infection.

Also the steroid drop should always be used in conjunction with a topical antifungal agent.

-ROLE OF MYDRIATIC-CYCLOPLEGIC AGENT:

a. It relieves ciliary spasm
b. It reduces intraocular inflammation
c. It prevents/ breaks posterior synechiae.

SIGNS OF IMPROVEMENT IN A CASE OF FUNGAL CORNEAL ULCER:

a) Reduction in size of the ulcer
b) Rounding of the perimeters of the lesion
c) Resolution of hypopyon
d) Resolution of satellite lesions
e) Reduction in intensity of stromal infiltration
f) Improvement in stromal and epithelial edema

SEQUELAE OF PROGRESSIVE INFECTIOUS KERATITIS:

- Corneal opacification
- Iris prolapse

- Descematocele formation- also called as keratocele. It results when the ulcer extends to the whole depth except for descemet's membrane which cannot withstand the intraocular pressure and herniates as a transparent vesicle through the ulcer.
- Corneal perforation- If perforation is small iris is gummed down to the opening resulting in synechiae. When whole cornea sloughs it results in false cornea and pseudo cornea. Corneal fistula results if perforation is opposite to the papillary area.
- Adherent leukomatous opacity
- Anterior staphyloma
- Secondary glaucoma
- Anterior capsular cataract
- Lens extrusion through perforation, subluxation and dislocation of lens.
- Hemorrhage – sudden perforation results in sudden decrease in IOP leading to rupture of vessels and cause vitreous hemorrhage, rupture of choroidal vessels leads to suprachoroidal, subretinal and expulsive hemorrhage.
- Endophthalmitis & panophthalmitis

VORICONAZOLE: It is a newer second generation triazole derived from fluconazole .it has broad spectrum activity against various fungi affecting the

eye . Marangon et al: voriconazole showed high susceptibilities for aspergillus, candida and fusarium.[32]

Mechanism of action:

it inhibits the enzyme cytochrome P450 dependant lanosterol 14 demethylase which is responsible for conversion of lanosterol to 14_demethyl lanosterol by binding to the active site of the enzyme and ligating the iron heme cofactor . this results in accumulation of 14- methyl sterols like lanosterol and depletion of ergosterol which alters both function and integrity of the fungal cell membrane.

It has a molecular mass of 349.32 Da that allows good corneal penetration, and therefore better ocular bioavailability.

Various case reports regarding the use of topical voriconazole in fungal keratitis:

1. Klont et al:reported a case of A 23-year-old man with *F. solani* keratitis who failed to respond to treatment with topical amphotericin B and itraconazole. The patient was then prescribed, concomitant IV and topical voriconazole followed by voriconazole orally and topically and this had a successful outcome.

2. Reis et al: in this report a 16-year-old girl diagnosed with non responding keratitis caused by *F. solani* . The patient was prescribed topical amphotericin B and fluconazole initially , followed by itraconazole later. However no response was noted.A significant improvement was noticed upon the addition of topical voriconazole to therapy,
3. Prats et al: reported a 19-year-old man diagnosed as a case of *S. apiospermum* keratitis. The infection resolved eoth topical voriconazole
4. Tu et al: reported two cases, one a 29-year-old man who received oral and topical voriconazole for *Fusarium* keratitis. In the second case, a 43-year-old woman received a combination of IV, topical, and intravitreal voriconazole for keratitis caused by *F. solani* that was associated with contact lens wear. In both of these cases, voriconazole was initially effective until it had to be discontinued because of severe hepatotoxicity. Patients were then switched to posaconazole as salvage therapy.
5. Polizzi et al: reported use of 2% voriconazole eye drops in a case of ulcer caused by fusarium solani
6. Jones et al: demonstrated that voriconazole was effective in a 52-year-old woman diagnosed as a case of *Aspergillus niger* keratitis who was initially treated with amphotericin B topically with no improvement. When the

patient was started on a combination of oral and topical voriconazole, the infection resolved after five weeks .

Intraocular penetration of systemic voriconazole:it is metabolized by liver. Its minimum inhibitory concentration of 0.5 microgram/ml is much less compared to other imidazoles.it has excellent oral bioavailability.after oral administration of voriconazole therapeutic aqueous and vitreous concentrations are achieved.

Shah et al, 2003 found the minimum inhibitory concentration of voriconazole was 0.5 microgram/ml a concentration lower than that of the other imidazoles.

Hariprasad et al: oral voriconazole reaches therapeutic MIC90 concentrations in the vitreous and aqueous against a wide range of organisms including aspergillus and candida.[31]

Systemic voriconazole has good intraocular penetration but it may result in adverse effects and also interactions with concomitant medications. Also systemically used it is expensive.

Voriconazole is phototoxic, it has been noted to be associated with transient visual disturbances. It has been reported in around 30% of patients in various clinical trials though studies have shown that there is no damage to any structure of the eye and no long term effect on vision.

Gao et al first demonstrated the safety of voriconazole in the eye: intravitreal voriconazole concentration upto 25 mg/ml did not produce any histolological changes or any electroretinographic changes in the rat retina.

Sponsel et al reported that topical voriconazole as a novel treatment for fungal keratitis

Jang et al reported that voriconazole is also effective against candida chorioretinitis.

Ozbek et al reported 1 case of alternaria species keratitis that showed dramatic improvement to 1% voriconazole.

Giaconi et al reported two cases caused by colletotrichum dematium and fusarium oxysporum which did not respond to treatment with 1% voriconazole eye drops.

LITERATURE RELATED TO INTRASTROMAL INJECTION OF VORICONAZOLE:

- Gaurav prakash, namrata Sharma et al did an interventional case series in three eyes of three patients with recalcitrant fungal keratitis non responding to topical antifungal medications and all three patients had faster reduction in size of corneal infiltration and complete resolution of

the ulcers was noticed within three weeks after intrastromal administration of voriconazole.[25]

- Punit K Singh, Ganesh Bhargava et al reported a series of two cases of deep seated fungal keratitis whose ulcer healed completely in both eyes after intrastromal administration of voriconazole over a period of twenty days. In this series only a mild improvement in visual acuity was noted because of the resultant scar which involved the central cornea.
- Tu, Elmer Y MD et al described the clinical presentation of 3 cases of *Alternaria* keratitis who presented with an indolent steroid-treated keratitis. They also had a history of recent cataract surgery, agricultural trauma, or contact lens wear. None of those patients responded to natamycin, and 1 among them also did not respond to topical and systemic voriconazole.these Patients responded rapidly to either topical fluconazole 0.02% or a combination of intrastromal voriconazole and topical caspofungin 0.5% and their final visual acuity ranged between 20/15 and 20/25
- Siatri , Heider et al observed dramatic therapeutic response in three patients with recalcitrant fungal keratitis as these patients failed to respond to 5% topical natamycin hourly and oral ketoconazole twice per day. One patient in this study underwent amniotic membrane

transplantation to seal the microperforation who had chemical burn superinfected with fusarium.[24]

- Namrata Sharma, prakash agarwal et al studied twelve patients with smear and / or culture positive fungal keratitis who did not respond to both topical and systemic antifungal therapy who were given one or more doses of intrastromal voriconazole injection. In this study, ten patients healed with scar formation while two corneas perforated and required therapeutic penetrating keratoplasty.[25]
- Vandana Jain, Nishikant Borse et al reported a case of a 50 year old woman who developed recalcitrant fungal tunnel infection following uneventful cataract surgery and found aspergillus flavus as the causative agent who did not respond to intensive medical treatment. Ulcer resolved completely after injection of intrastromal voriconazole.
- Namrata Sharma, Jacob Chaco et al did a randomized controlled clinical trial involving forty eyes of forty pstients with smear / culture proven fungal keratitis not responding to natamycin topically for two weeks comparing topical versus intrastromal voriconazole and concluded that intrastromal injection did not offer a beneficial effect when compared with topical therapy. In this study 19 patients who received topical

voriconazole and 16 patients who received intrastromal voriconazole responded well to therapy.[26]

- Stacy Bang , Erica Edell et al reported the use of both topical and intrastromal voriconazole in three eyes with resistant acanthamoeba keratitis which was resistant to treatment with chlorhexidine.

SIGNS OF NON HEALING CORNEAL ULCER: any corneal ulcer is considered to be a non healing one if it shows signs and symptoms of progression in spite of maximal therapy(based on culture and sensitivity in cases of infectious ulcer). Symptoms include increasing pain, redness, discharge, photophobia and decreasing visual acuity. Signs are as follows:

- Increase in size of the ulcer
- Necrotic material covering the ulcer bed with no granulation tissue
- Increasing density of the stromal infiltrate, extending deeper than the mid stroma
- No well demarcation of the ulcer with increasing corneal haze and stromal edema.
- Increasing hypopyon and increasing anterior chamber reaction
- Absence of peripheral corneal vascularisation.

CAUSES OF NON HEALING CORNEAL ULCER:it can be classified as both local and systemic causes. Local causes include :

- eyelid and eyelash abnormalities like ectropion, entropion, trichiasis, lagophthalmos, blepharitis, meibomitis which does not allow the ulcer by either mechanical irritation, exposure, tear film instability and contributing infection.
- Infections of the lacrimal sac both acute and chronic dacryocystitis
- Conjunctival surface disorders like stevens Johnson syndrome, foreign body in the tarsal conjunctiva, conjunctival concretions.
- Causes of dry eye like primary keratoconjunctivitis sicca and sjogren's syndrome as tear film forms an important defense mechanism by mechanical washing out the infecting agent, anti bacterial action of tear film constituents and by supplying oxygen to the cornea.
- Neurotrophic keratitis as decreased corneal ulceration leads to loss of epithelial integrity and thereby leading on to epithelial and stromal ulceration.
- Secondary glaucoma as increased intraocular pressure affects the corneal nutrition by causing impairment of diffusion of fluid through the cornea.

Systemic causes include diabetes mellitus causing delayed epithelial healing, malnutrition, immunosuppression, chronic alcoholism. Common causes of non healing ulcer include poor compliance of the patient, inappropriate antimicrobials and no proper microbiological work up being done.

PROGNOSIS OF FUNGAL KERATITIS : It varies depending on the depth and size of the lesion and the causative organism. small superficial infections respond well to topical therapy generally. Deep stromal infections and with concomitant scleral or intraocular involvement are much more difficult to eradicate.

One prospective, interventional study involving 115 cases with fungal keratitis assessed treatment outcomes and prognosis after 1 month of topical natamycin use.[1] 52 were considered treatment successes, 27 patients had slow-healing ulcers, and 36 were reported as treatment failures. Multivariate analysis highlighted that the three factors associated with treatment failure were large ulcer size (greater than 14 mm^2), the presence of a hypopyon, and *Aspergillus* as the causative organism. Surgical therapy can prove successful when medical treatment fails or can be tried as a secondary measure for visual rehabilitation.

ROLE OF SURGERY IN FUNGAL KERATITIS:

At present surgery is seldom needed during the acute phase of the infection although it may be required later to restore vision if significant corneal scarring occurs. About one third of fungal infections require surgical intervention as a result of treatment failures or perforation.forst Surgical management can be employed in one of the following forms:

i. Tissue adhesives: cyanoacrylate glue is used for perforation less than 1 mm or in cases of descematocele
ii. Debridement:it aids in reducing the microbiological and antigenic load. It is the simplest type of surgical intervention and can be performed under slit lamp under topical anaesthesia.It also facilitates drug penetration, provides material for culture and prevents early epithelisation over areas of stromal infiltrarion.
iii. Cauterization: it can be done by either thermal or chemical methods. The chemicals involved in cautery include 20% trichloroacetic acid or 100% carbolic acid or iodine.it destroys the organism but leaves behind a

permanent opacity thereby not used in central ulcers. It is contraindicated in thinned out corneas and perforated ulcers.

iv. Conjunctival flap: it is coupled with debridement in cases of non healing ulcers. It serves as a structural support and provides increased antibodies by increasing blood supply.

v. Superficial keratectomy aids in removing the devitalized tissues and fungal elements.

vi. Tectonic graft

vii. Penetrating keratoplasty: it is the ideal procedure in cases of progressive non healing ulcers. The main aim here is to control the infection and maintain the structural integrity.Note that the trephination should extend 1-2 mm beyond the infiltrate.the indications of penetrating keratoplasty in keratomycosis are:

- Progressive ulceration
- Perforated cornea
- Impending perforation
- Extensive drug toxicity
- Uncontrolled inflammation

Following penetrating keratoplasty topical antifungals should be used to prevent recurrence.

COMPLICATIONS OF KERATOPLASTY:

- Intraoperative complications include: sclera perforation, trephination related problems, retained descemets membrane, endothelial damage, vitreous loss, intraocular hemorrhage
- Early Post operative complications include wound leak, papillary block, postoperative inflammation, raised intraocular hemorrhage, suture related infiltrates and vascularisation
- Late post operative complications include astigmatism, graft infection, graft rejection and glaucoma.

PART-2

AIM OF THE STUDY:

1. To evaluate the role of intrastromal voriconazole in fungal corneal ulcers refractory to topical antifungals.
2. To identify the fungal species causing keratitis
3. To determine the fungal species most responsive to voriconazole.
4. To determine the nature of injurious agents in causing keratitis.

MATERIALS AND METHODS:

The study was conducted in Regional Institute of Ophthalmology. Patients with corneal ulcer were followed for a period of one year.464 cases of corneal ulcer were treated in our hospital. Out of the fungal culture positive cases 25 cases were selected for the study who did not respond to topical antifungals for more than one week and in whom other causes of non healing corneal ulcer(systemic causes like uncontrolled diabetes, immunosuppressed state and local causes like entropion, ectropion, trichiasis, lagophthalmos, lacrimal sac infections, ocular surface disorders) were ruled out. These patients were treated with 50 microgram/0.1 ml of intrastromal voriconazole injection.

INCLUSION CRITERIA:

- Patients in age group of ten years and above of either sex.
- Patients presenting with corneal ulcers of proven fungal etiology by KOH mount and who did not show why improvement on topical antifungals after one week.

EXCLUSION CRITERIA:

- Patients aged less than ten years.

- Patients with impending perforation/ perforated corneal ulcers.
- Patients presenting with associated bacterial corneal ulcers/ peripheral ulcerative keratitis.
- Patients presenting with healing corneal ulcers.
- Pregnant females
- Patients with uncontrolled diabetes mellitus, other systemic diseases and immunocompromised state.
- Patients presenting with corneal ulcers with involvement of adjacent sclera and concomitant endophthalmitis.

COURSE OF THE STUDY:

- Patients presenting with corneal ulcer were subjected to detailed ophthalmological examination including visual acuity, slit lamp biomicroscopy- the findings to be noted in slit lamp include: the area and density of infiltration, size and depth of ulceration, size of epithelial defect, degree of stromal edema, anterior chamber reaction and sclera involvement,syringing of the nasolacrimal duct, routine smear studies under sterile conditions on precleaned glass slides-one for 10% KOH mount to look for fungal elements and another slide for Gram stain to look for bacterial pathogens. Additionally, the corneal scraping was inoculated in Sabouraud's agar and kept at room temperature too. Random blood sugar and urine sugar testing too was done to rule out diabetes mellitus.
- The fungal species were confirmed by growth in culture media. Species identification was done with lactophenol cotton blue for 25 culture positive cases.
- Sociodemographic data of these patients and the information related to risk factors too was noted like history of any trauma.
- Those patients aged more than ten years in whom initial smear studies and clinical features favour fungi as the causative agent were treated with 5%

natamycin , fluoroqinolone antibiotics to prevent superadded bacterial infection and adjuvant medications such as atropine eye drops. those patients in whom signs and symptoms of progression were noted despite one week of topical medication and in whom both local and systemic causes of non healing ulcer were ruled out were treated with 50 microgram/0.1 ml of intrastromal injection of voriconazole.

- Informed consent was obtained from the patients before administration of the drug
- Method of intrastromal administration: 1 mg of voriconazole containing vial was reconstituted with 2 ml of distilled water. The reconstituted solution was loaded in syringe with 26 gauge bent needle.Under total aseptic precautions,under topical anaesthesia, 0.1 ml of the preloaded drug was administered under the operating microscope.The needle was inserted obliquely from clear area to the ulcer at *mid stromal* level.*Multiple injections* were given circumferentially to ensure formation of drug depot around the entire lesion.
- Poststromal injection all patients were continued on topical antifungals.
- Patients were examined every day under the slit lamp and response to therapy documented recording the size of the ulcer and the best corrected visual acuity

- All these patients were followed up for a period of 30 days.
- When patients did not show any improvement and had gross structural damage or developed perforation or signs of impending perforation were taken up for urgent therapeutic keratoplasty.

ASSESMENT OF PARAMETERS: the following parameters are assessed- visual acuity, size of the infiltration , hypopyon and associated features .

1. Symptomatic improvement in the patient
2. Decrease in epithelial defect and infiltration
3. Improvement in patient's best corrected visual acuity
4. Resolution of hypopyon if present

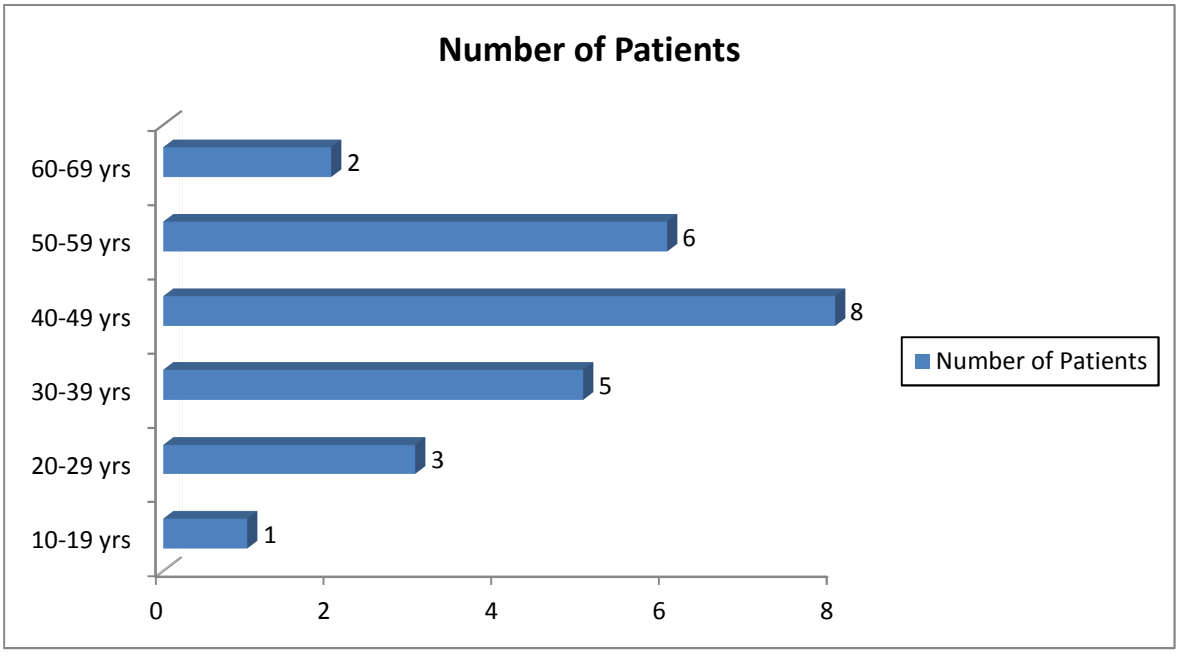
ANALYSIS:

1.AGE DISTRIBUTION:

Table 1: age wise distribution

AGE	NUMBER	PERCENTAGE
10-19	1	4%
20-29	3	12%
30-39	5	20%
40-49	8	32%
50-59	6	24%
60-69	2	8%

Chart 1- agewise distribution

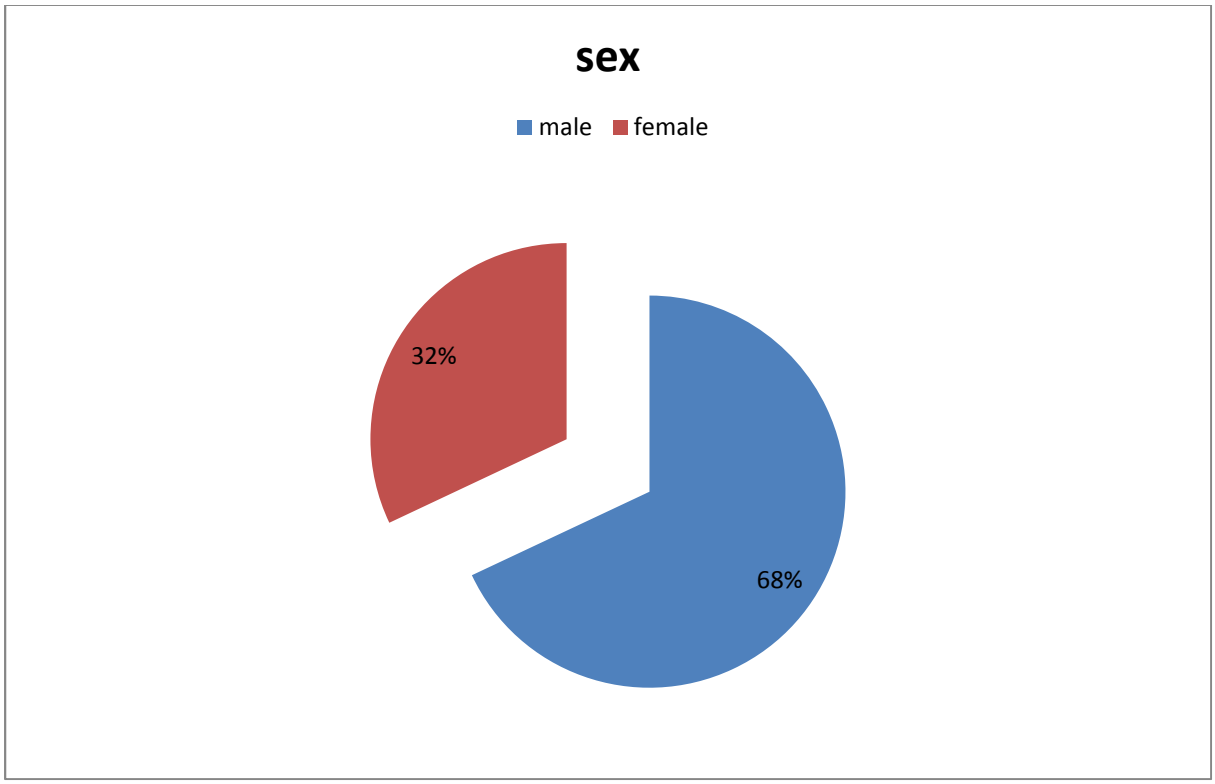


SEX DISTRIBUTION:

TABLE 2: SEX DISTRIBUTION

S.NO	SEX	NUMBER	PERCENTAGE
1	MALE	17	68%
2	FEMALE	8	32%

CHART 2: SEX DISTRIBUTION

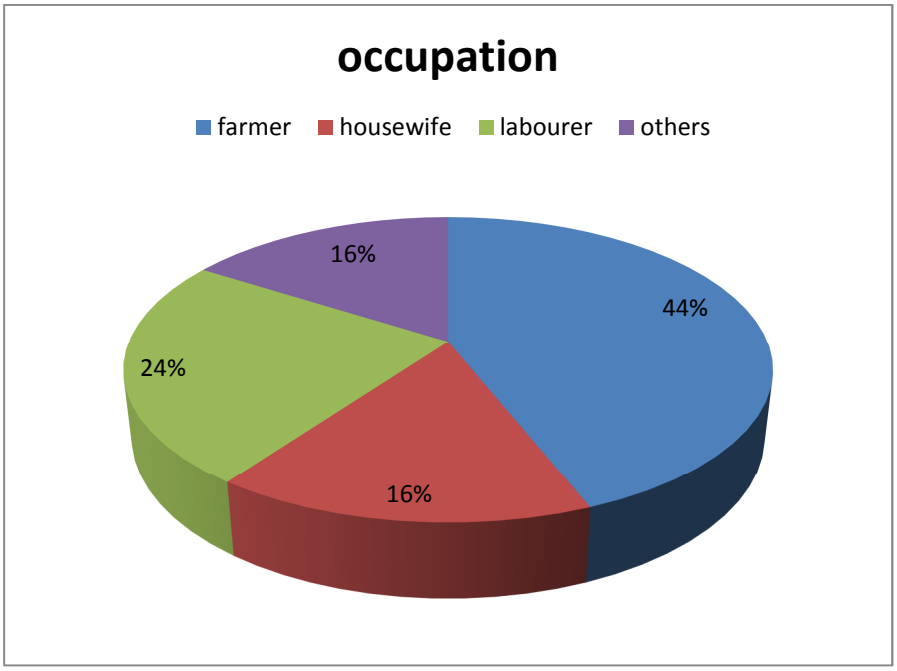


OCCUPATION:

Table 3: occupation wise distribution

S.NO	OCCUPATION	NUMBER	PERCENTAGE
1	farmer	11	44%
2	Housewife	4	16%
3	Labourer	6	24%
4	Others	4	16%

Chart 3: occupation wise distribution

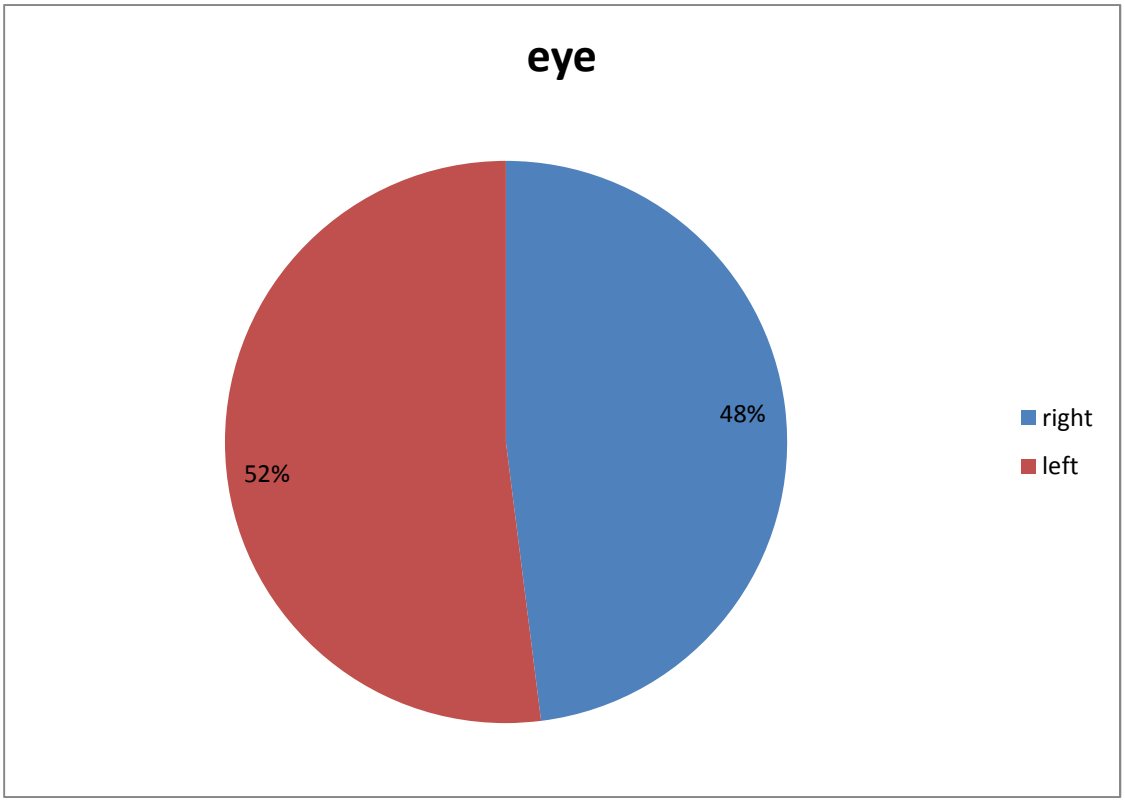


EYE LATERALITY:

TABLE 4: EYE LATERALITY

S.NO	EYE	NUMBER	PERCENTAGE
1	RIGHT EYE	12	48%
2	LEFT EYE	13	52%

CHART 4: EYE LATERALITY

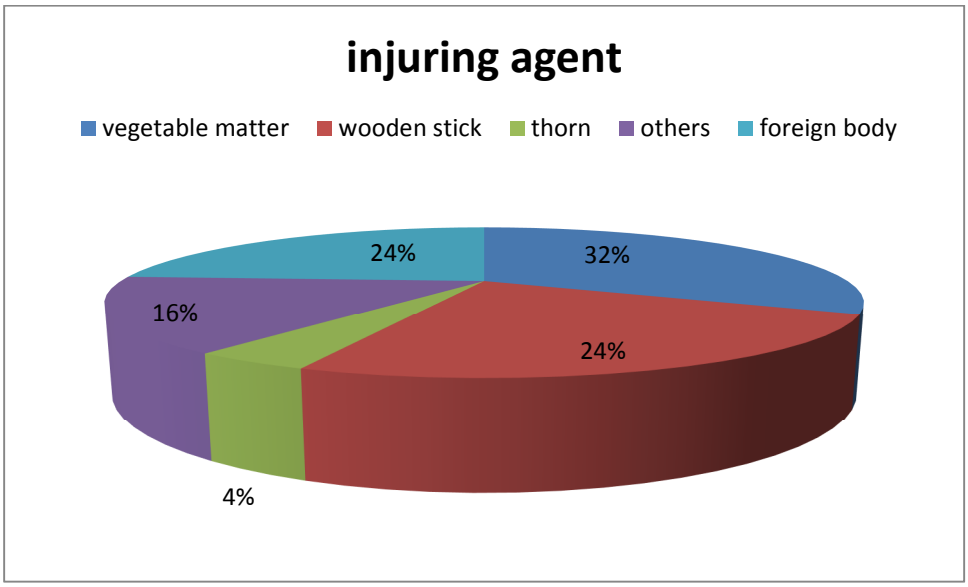


INJURING AGENT:

TABLE 5: injuring agent

S.NO	NATURE OF INJURING AGENT	NUMBER	PERCENTAGE
1	Vegetable matter	8	32%
2	Wooden stick	6	24%
3	Thorn	1	4%
4	Foreign body including mud particle, cement	3	24%
5	Others/ no injuring agent	4	16%

Chart 5: injuring agent

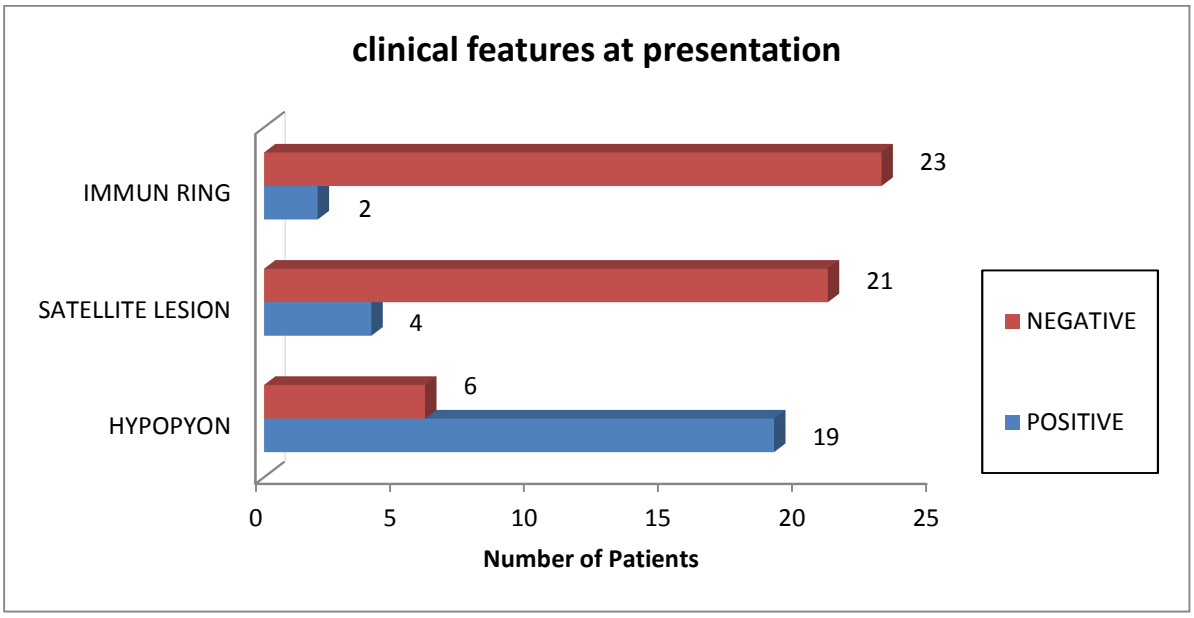


CLINICAL FEATURES AT PRESENTATION:

TABLE 6: CLINICAL FEATURES AT PRESENTATION:

S.NO	CLINICAL FEATURE	NUMBER	PERCENTAGE
1	HYPOPYON	19	76%
2	SATELLITE LESION	4	16%
3	IMMUNE RING	2	8%

CHART:6- CLINICAL FEATURES AT PRESENTATION

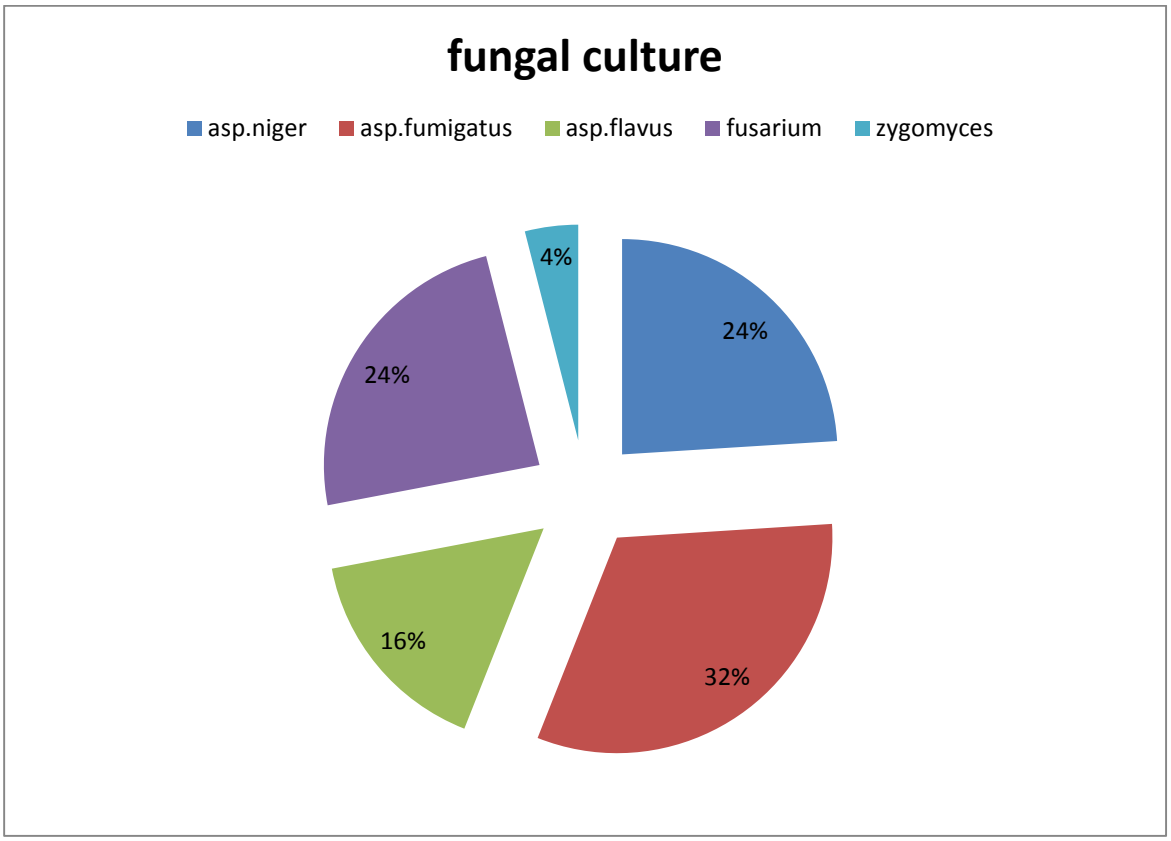


FUNGAL SPECIES ISOLATED ON CULTURE:

Table 7 : fungal species isolated on culture

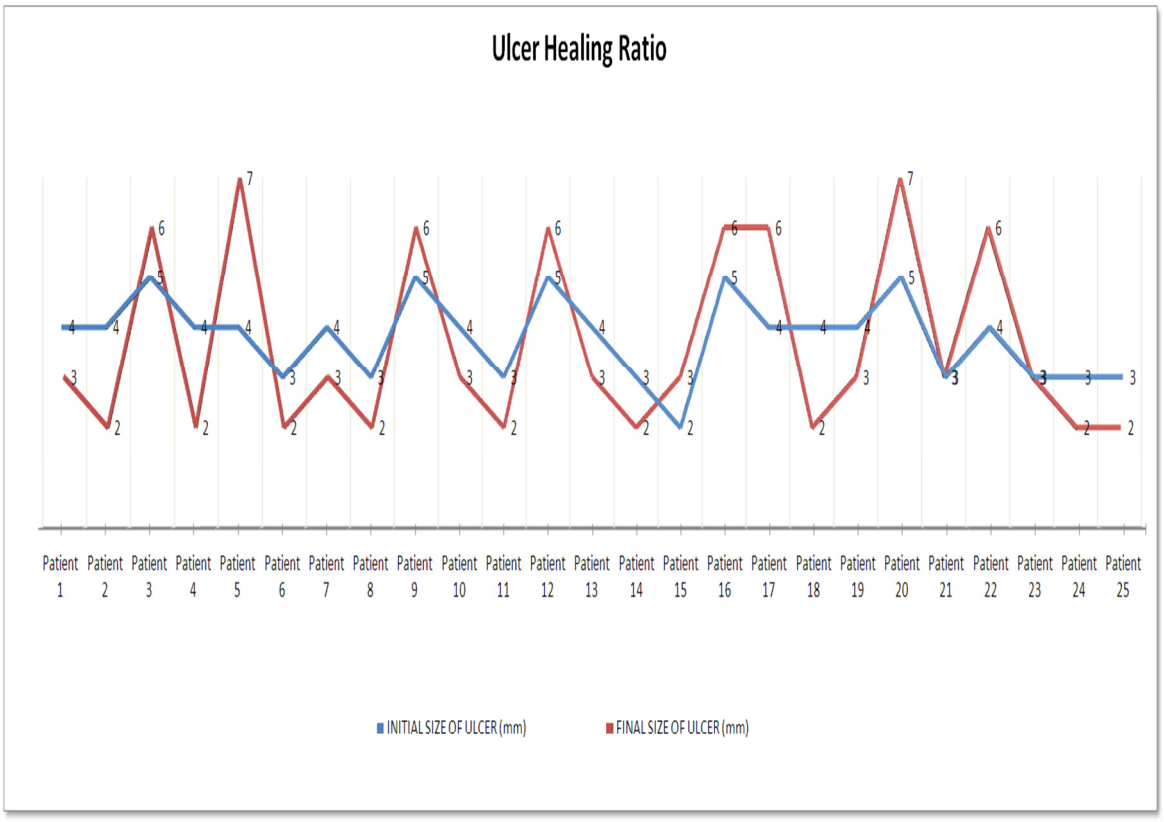
S.NO	FUNGAL SPECIES	NUMBER	PERCENTAGE
1	Aspergillus niger	6	24%
2	Aspergillus fumigates	8	32%
3	Aspergillus flavus	4	16%
4	Fusarium	6	24%
5	Zygomycetes	1	4%

Chart 7: fungal species isolated on culture



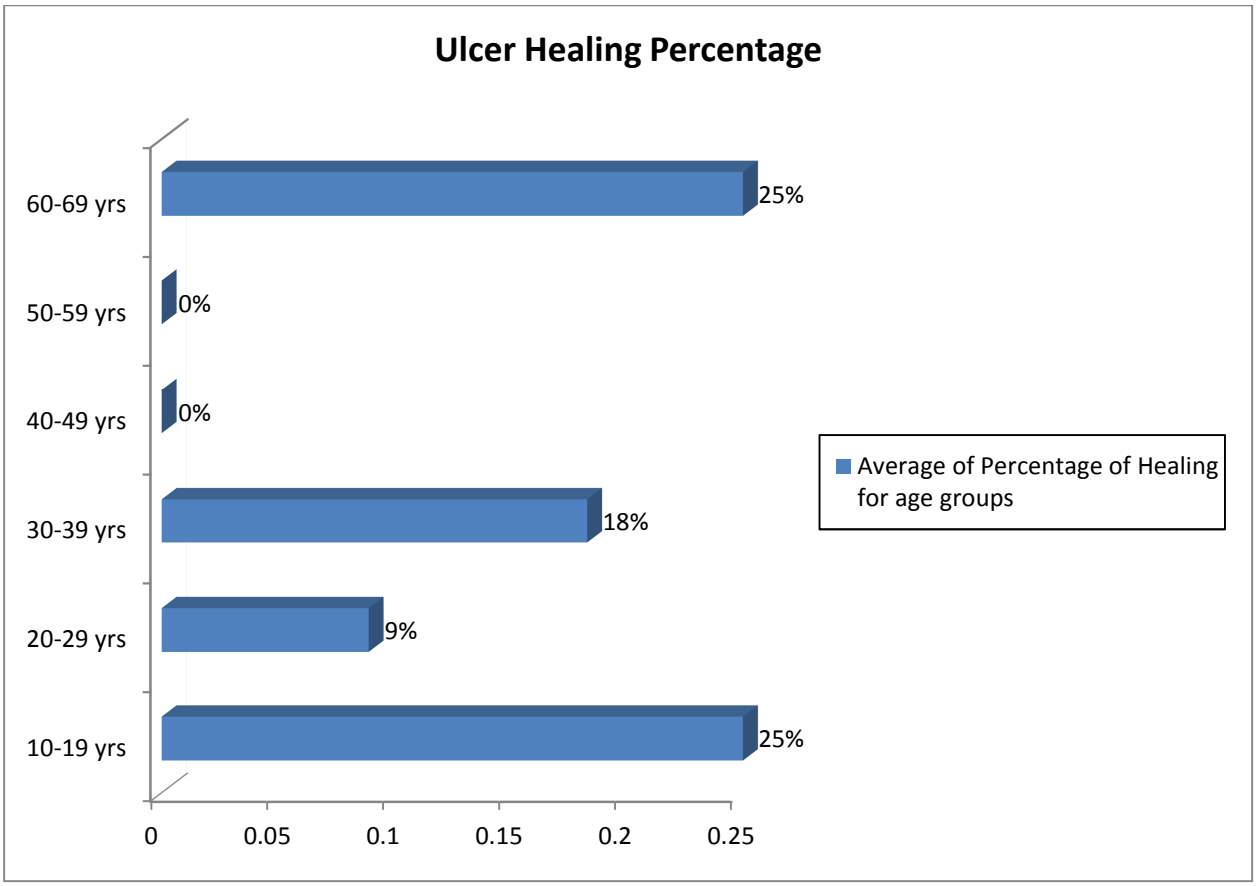
SIZE OF THE ULCER

CHART 8: SIZE OF THE ULCER PRE AND POST TREATMENT



The healing ratio is taken for one patient at a time.

The x-axis values represent the patient numbers.


Ulcer Healing Percentage
60-69 yrs
50-59 yrs
40-49 yrs
30-39 yrs
20-29 yrs
10-19 yrs
25%
0%
0%
18%
9%
25%
0
0.05
0.1
0.15
0.2
0.25
Average of Percentage of Healing for age groups

VISUAL ACUITY:

TABLE: 9: VISUAL ACUITY PRE TREATMENT

Visual acuity	No of cases	% of total cases
PL+ to <= 1/60	15	60%
1/60 to <=3/60	3	12%
3/60 to <=6/60	7	28%
More than 6/60	Nil	0%

TABLE 10: VISUAL ACUITY POST TREATMENT

Visual acuity	No of cases	% of total cases
PL+ to <= 1/60	12	48%
1/60 to <=3/60	0	0%
3/60 to <=6/60	7	28%
More than 6/60	6	24%

CHART: 9

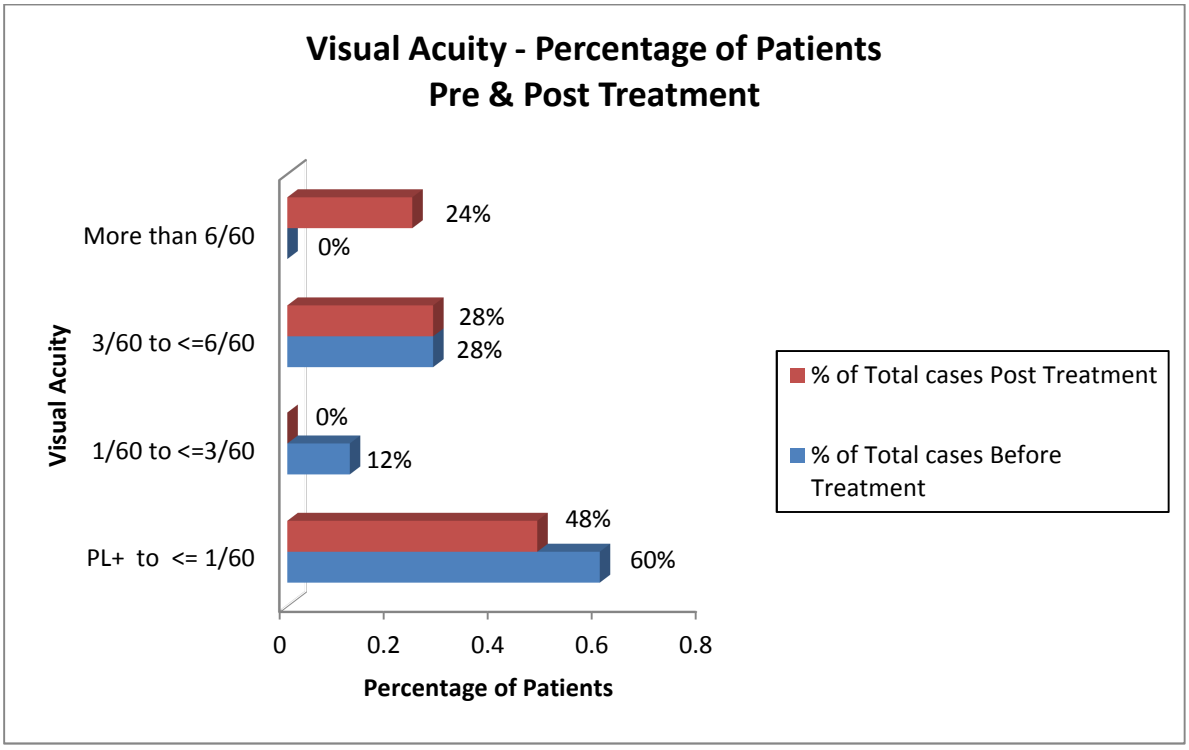


CHART 10:

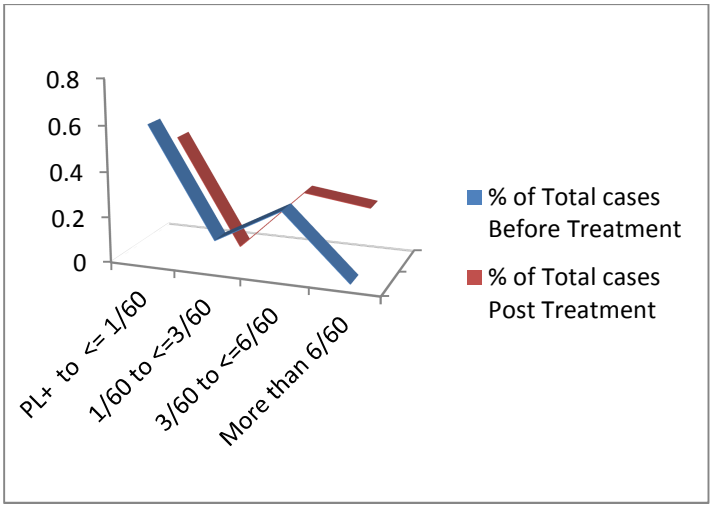


CHART:11

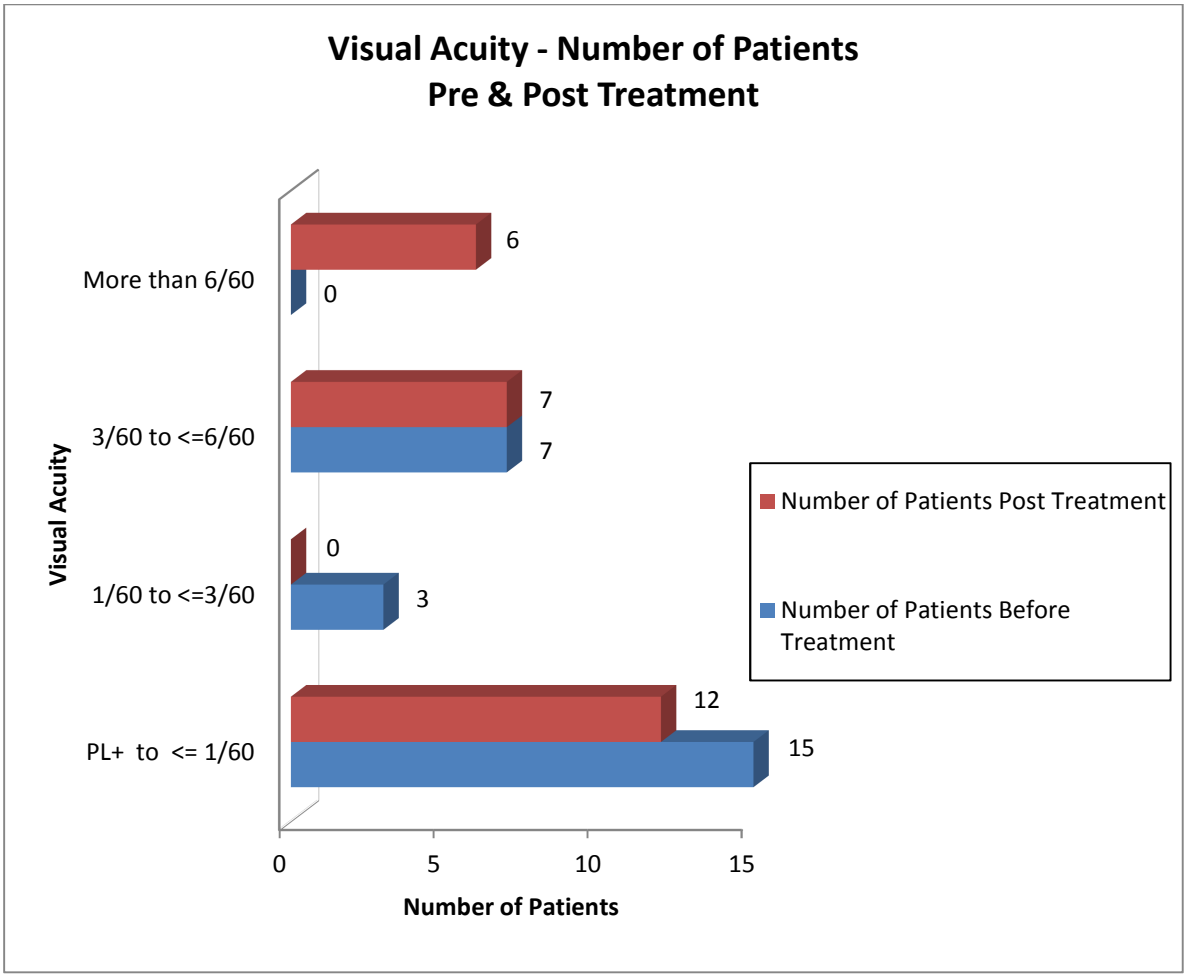

Visual Acuity - Number of Patients
Pre & Post Treatment
More than 6/60
3/60 to <=6/60
1/60 to <=3/60
PL+ to <= 1/60
Visual Acuity
6
0
7
7
0
3
12
15
0
5
10
15
Number of Patients
Number of Patients Post Treatment
Number of Patients Before Treatment

CHART 12: RESPONSE OF DIFFERENT FUNGAL SPECIES TO INTRASTROMAL VORICONAZOLE:

S.NO	SPECIES	NUMBER OF PATIENTS WHO RESPONDED WELL	PERCENTAGE
1	Asp. Fumigates	6 out of 8	75%
2	Asp. Niger	4 out of 6	66.66%
3	Asp. Flavus	1 out of 4	25%
4	Fusarium	5 out of 6	83.33%
5	Zygomycetes	1 out of 1	100%

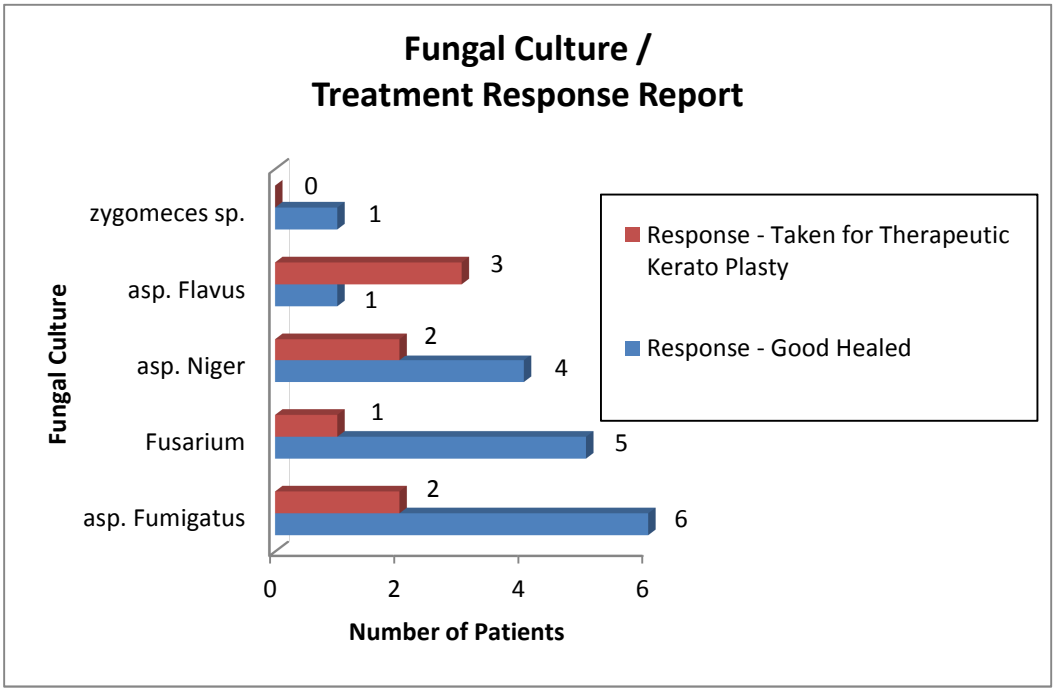
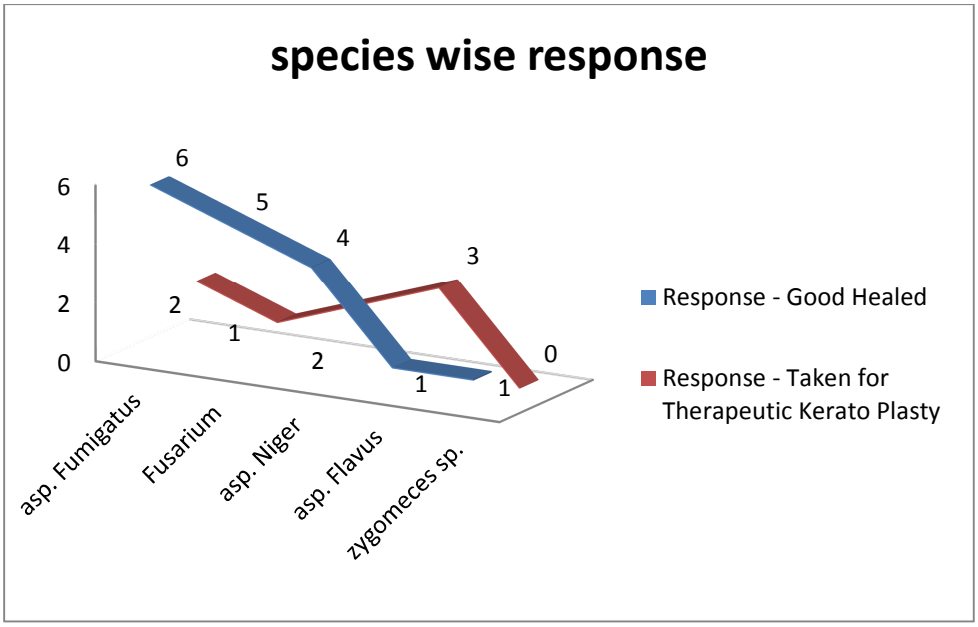


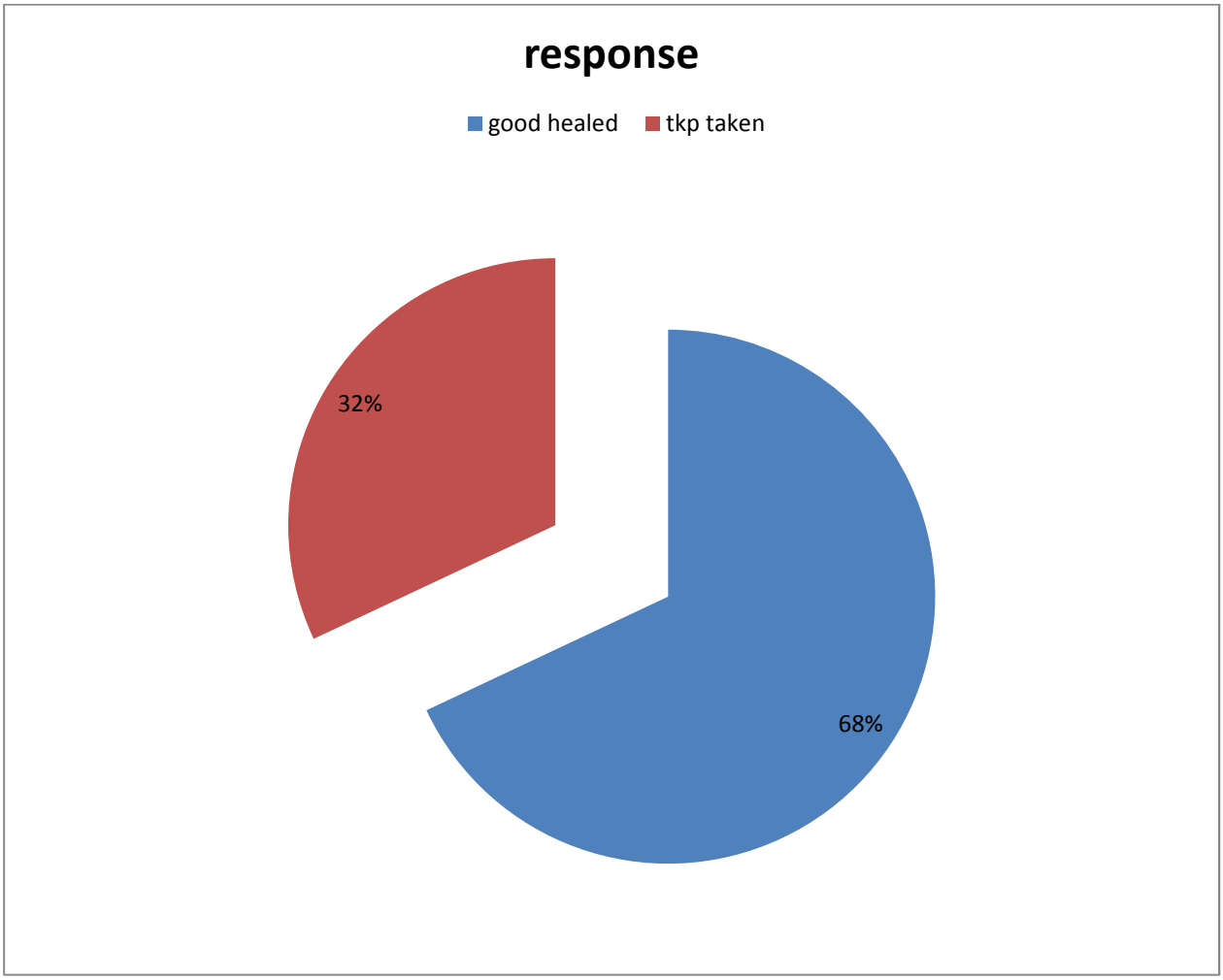
CHART 14:


species wise response
6
4
2
0
6
5
4
3
2
1
2
1
1
0
asp. Fumigatus
Fusarium
asp. Niger
asp. Flavus
zygomeces sp.
Response - Good Healed
Response - Taken for Therapeutic Kerato Plasty

RESPONSE:

S.NO	RESPONSE	NUMBER	PERCENTAGE
1	HEALED	17	68%
2	TAKEN FOR TKP	8	32%

CHART 15: RESPONSE TO INTRASTROMAL VORICONAZOLE



Among 25 patients 8 were taken for therapeutic keratoplasty as they developed signs of impending perforation/ perforated.

ADVERSE EFFECTS:

None of the patient developed any side effect to intrastromal injection of voriconazole.

DISCUSSION:

Treating a case of fungal keratitis is a challenge for the ophthalmologist as the penetration of many antifungal agents commonly used remains inadequate. In this study we have reported the use of voriconazole in twenty five patients who did not show any improvement to topical antifungal agents even after one week of treatment.

Voriconazole is a broad spectrum antifungal agent though systemic administration has good intraocular penetration it is very costly. Therefore an economical and efficient strategy of using voriconazole in the management of fungal keratitis is needed which can be achieved by intrastromal route which we have employed in this study.

In our study 52% of cases belonged to male sex. This slight preponderance could be due to males being more involved in outdoor activities and therefore prone for injury.This has been pointed out by Whitcher in an article named “prevention of corneal ulcer”. Also this is in accordance to several studies reported earlier like the Madurai study by Srinivasan et al which had male: female ratio as 1.6:1. Here in our study the ratio was 1.08.[37]

With respect to age distribution 32 % of cases fall in the age group 40-49 years . this is in conformity with studies like done by Laspina et al[40] where it was noted that the greatest incidence was in the age group of 30-59 years As per review of 1352 patients in south india by Gopinathan, Usher et al males are significantly affected more with 64.4% of cases in the younger age group (16-49 years) .

Majority of cases belonged to lower socioeconomic group with farmers being predominantly involved (44% of cases in our study) like the one done by Rahman et al.

History of injury was present in 84 % of cases with vegetable matter injury being the common inciting agent. Trauma was the major causative agent in fungal corneal ulcers as per study by Upadhyay MP, Karmacharya PC et al[39]. vegetable matter injury has been reported in many studies before like the one conducted by Bharathi et al .

Fungal keratitis has a varied clinical presentation. Hypopyon was present in 76% of cases, satellite lesions in 16% and immune ring was seen in 8% of patients.A three-year study by M Jayahar Bharathi, R Ramakrishnan, S Vasu et al [5]at Aravind Eye Care System, Tirunelveli, Tamil Nadu, reported that the clinical picture of fungal keratitis is pleomorphic. It varies from individual to individual, and largely depends on the type of causative fungus, invading pathogen's severity, liberation

of toxins, age of the patient and resistance of the host tissue. The unique features noted were a dry, raised lesion with a feathery or hyphate border and/ or as stromal infiltration. Satellite lesions were seen in 10% patients in that study. Other lesions that helped in diagnosis were cheesy hypopyon in 55.6% patients, immune ring in 1.37% patients, corneal perforation in 1.37% and posterior corneal abscess in 1% of the patients studied. The sensitivity and specificity of clinical diagnosis of fungal keratitis was 94.1% and 94.58% respectively made by an ophthalmologist using slitlamp biomicroscope

In our study Among the species identified, the most common species were aspergillus fumigatus. This was similar to the study results by Chowdhary, Anuradha Singh, Kirt et al where The spectrum of fungi isolated were *Aspergillus* species in 78 (41%) followed by *Curvularia* species in 55 (29%).As per study by Vajpayee et al fusarium was reported to be the most frequent cause of fungal keratitis, causing up to 32% of these infections.

Size and depth of the ulcer have a role in determining the outcome to treatment as superficial and smaller ulcers responded well to the treatment compared to those which were larger and involving the inner thirds of stroma.

In our study 60% of the total cases presented with visual acuity of less than 1/60. Following treatment with intrastromal voriconazole 48% of cases had visual acuity of less than 1/60.

Five out of six fusarium patients responded well with voriconazole injection given intrastromally.

Of twenty five cases studied here, 68% had good response which was documented by decrease in ulcer size and improvement in visual acuity and 32% were taken for therapeutic keratoplasty.

SUMMARY:

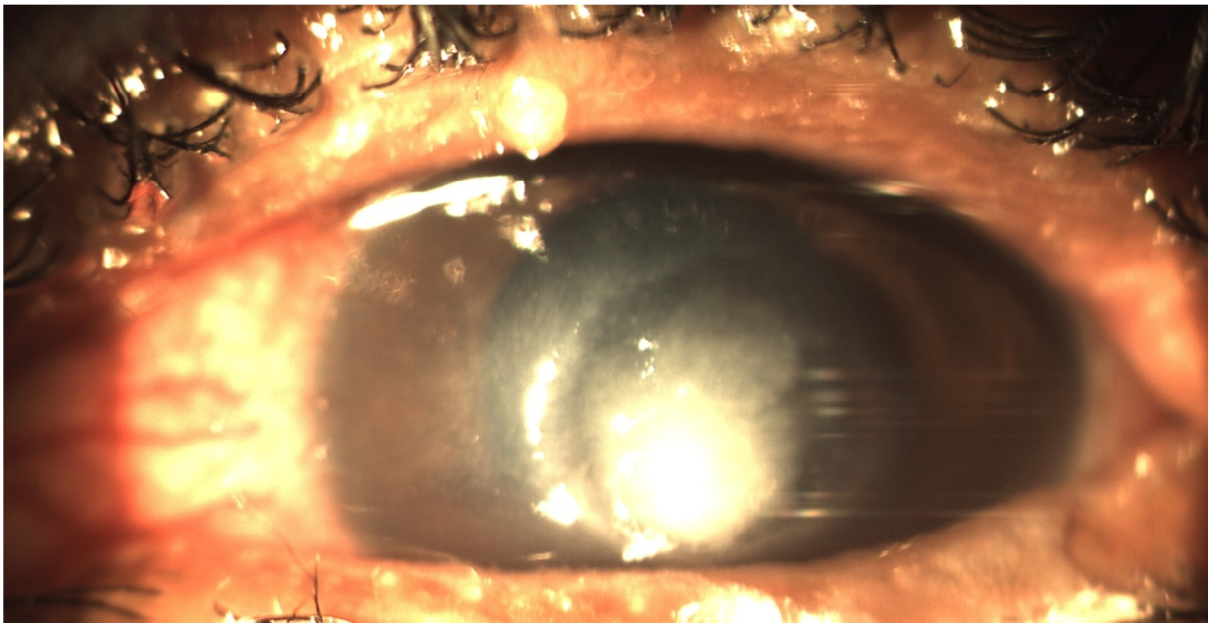
- Majority of the patients were males.
- Common age group affected 40-49 years
- Most common injuring agent was found to be vegetable matter(32% of cases) followed by wooden stick
- Occupation- farmers were commonly involved
- The most common organism isolated among 25 culture positive cases were aspergillus fumigates
- Hypopyon was present in 76% of patients indicating the severity of ulcers at presentation .
- Most of the patients fall into grade two and three severity as 68% of patients presented with ulcers greater than 4 mm area.
- The species which had shown better response to intrastromal voriconazole was fusarium and zygomycetes.
- 17 cases responded well to intrastromal voriconazole injection.
- 8 cases did not respond to treatment and underwent therapeutic keratoplasty.
- None of the patients developed any side effects to the voriconazole drug given intrastromally.

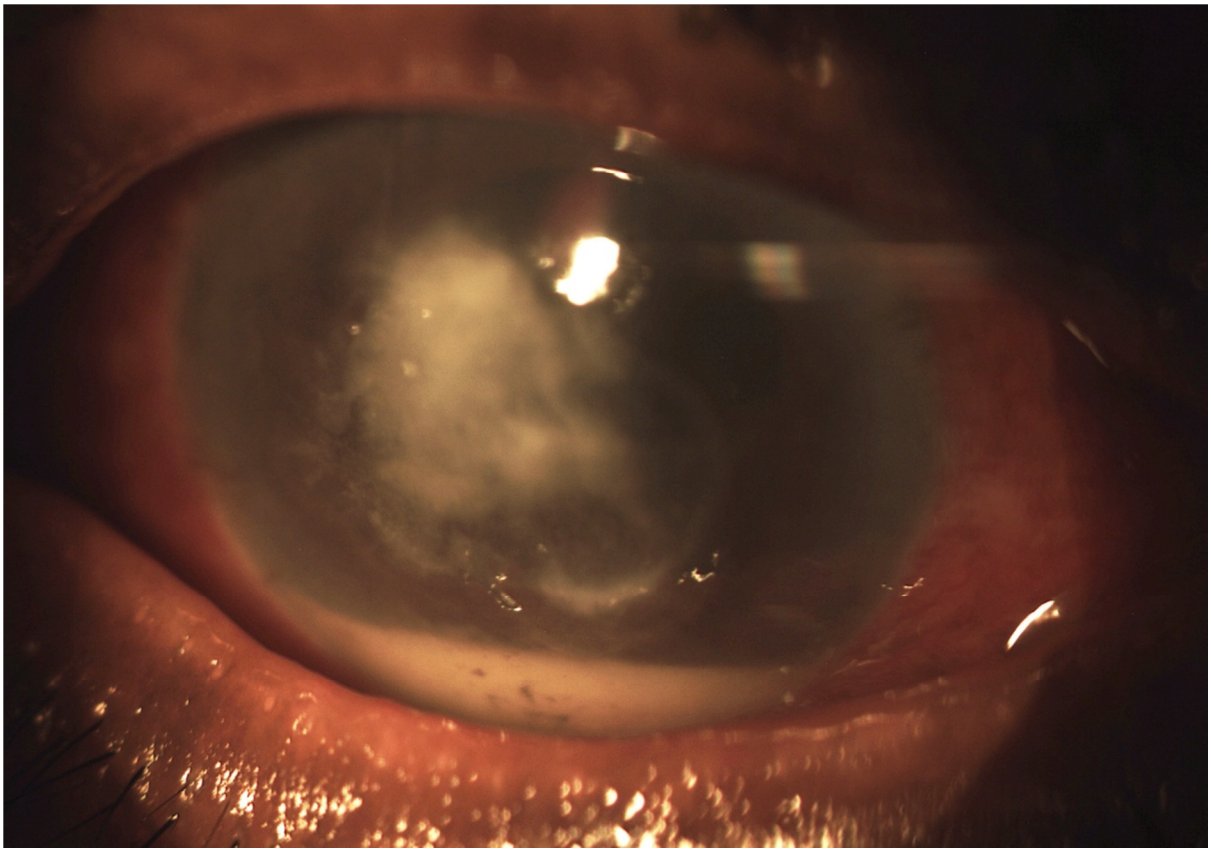
- Decrease in ulcer size and improvement in visual acuity was noted in responded cases. The improvement in visual acuity is not very significant here as the ulcer heals to form a scar obscuring the visual axis.

CONCLUSION:

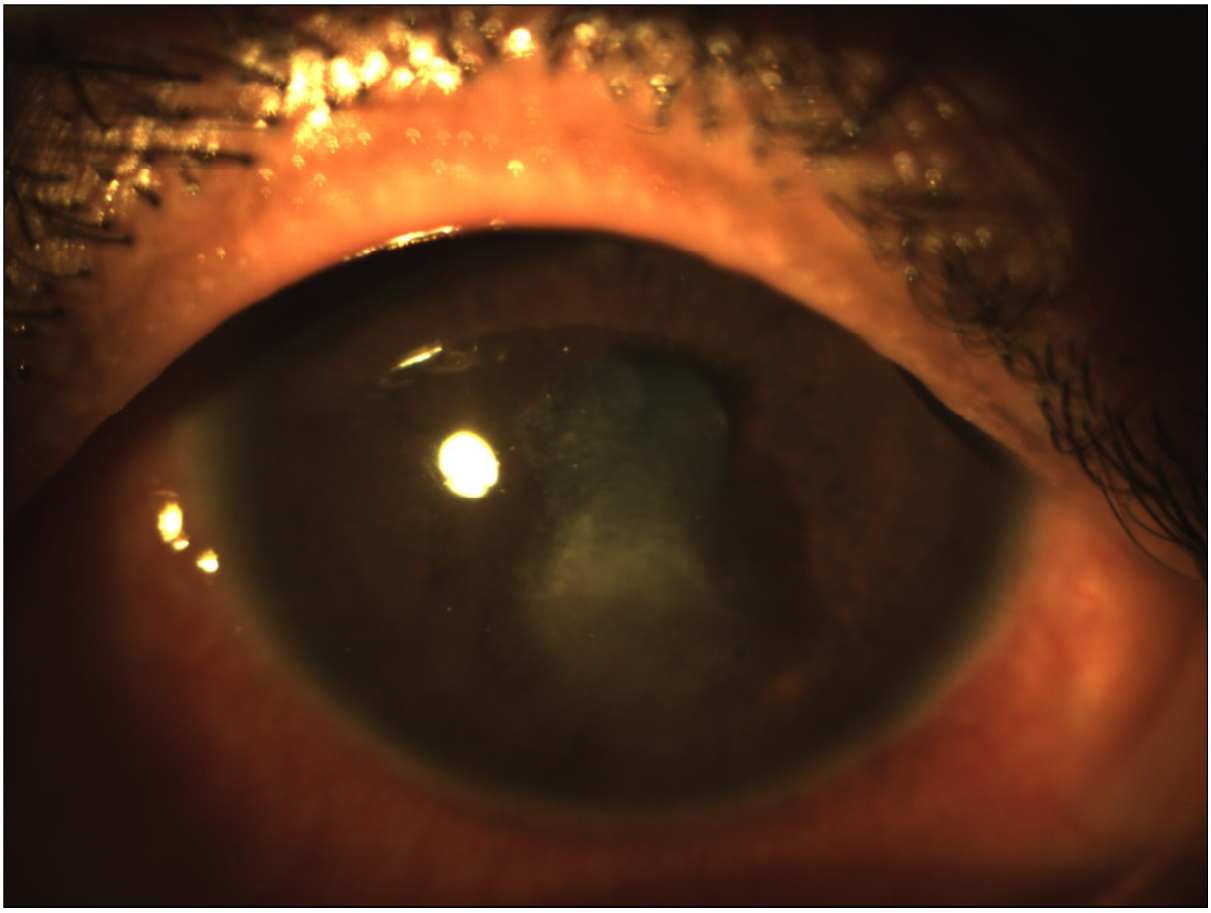
- ✓ Modalities of targeted delivery like intrastromal injections can improve the clinical outcomes of deep seated fungal corneal ulcers unresponsive to topical antifungals.
- ✓ Intrastromal administration of voriconazole might be a safe and cost-effective method of providing higher concentration of the drug.
- ✓ It also helps in delaying the progression of ulcer when the patient is waiting for donor cornea for TKP.

DRY LOOKING FUNGAL CORNEAL ULCER WITH FEATHERY MARGINS

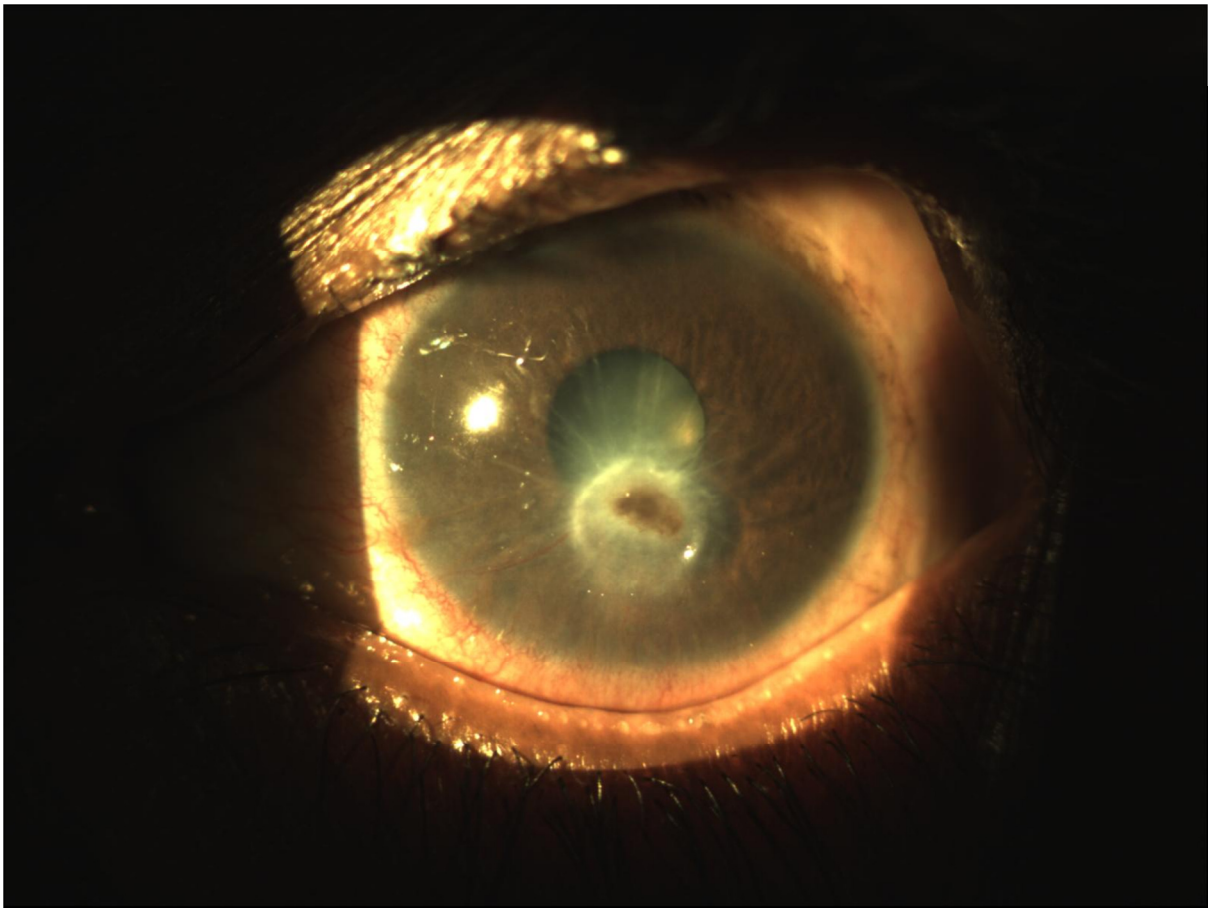




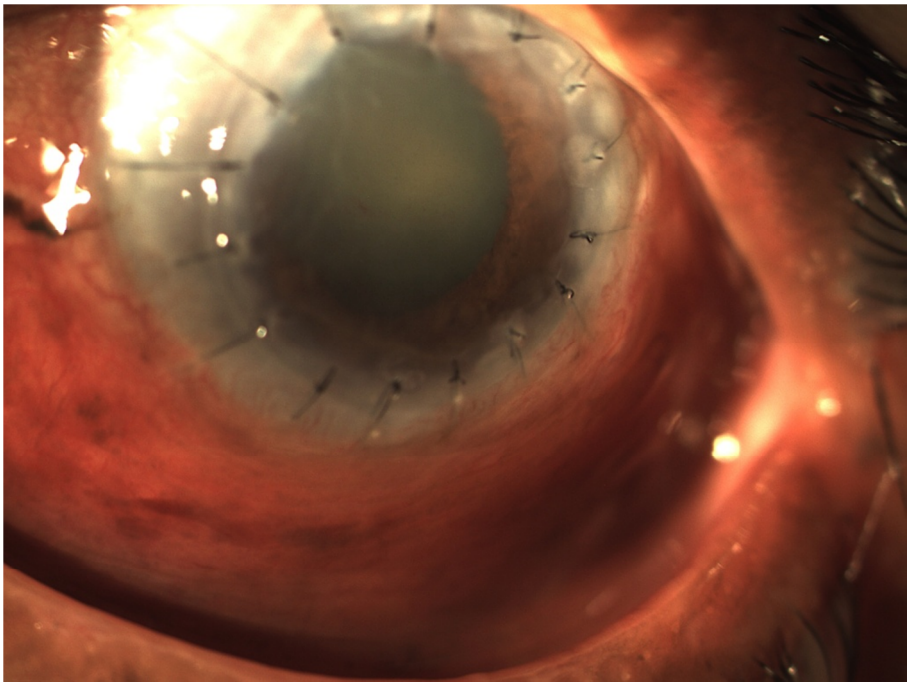
FUNGAL ULCER WITH FEATHERY MARGINS AND THICK HYPOPYON(patient no 5)



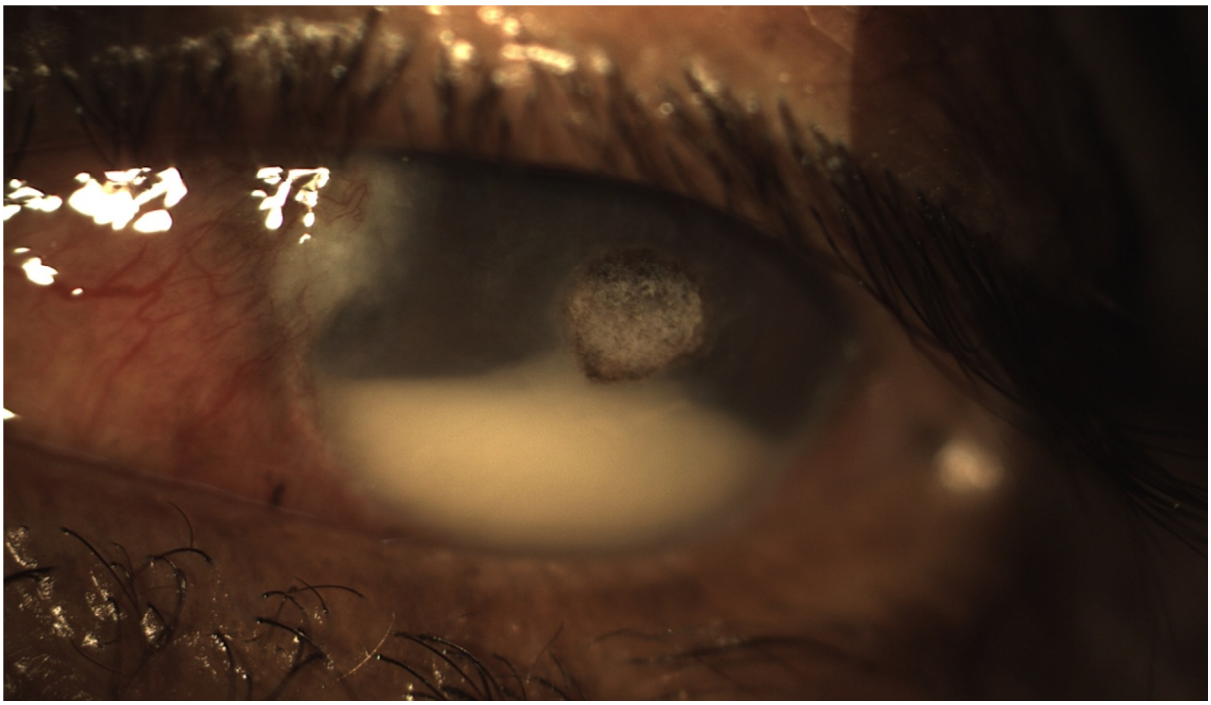
HEALING CORNEAL ULCER(patient no: 14)



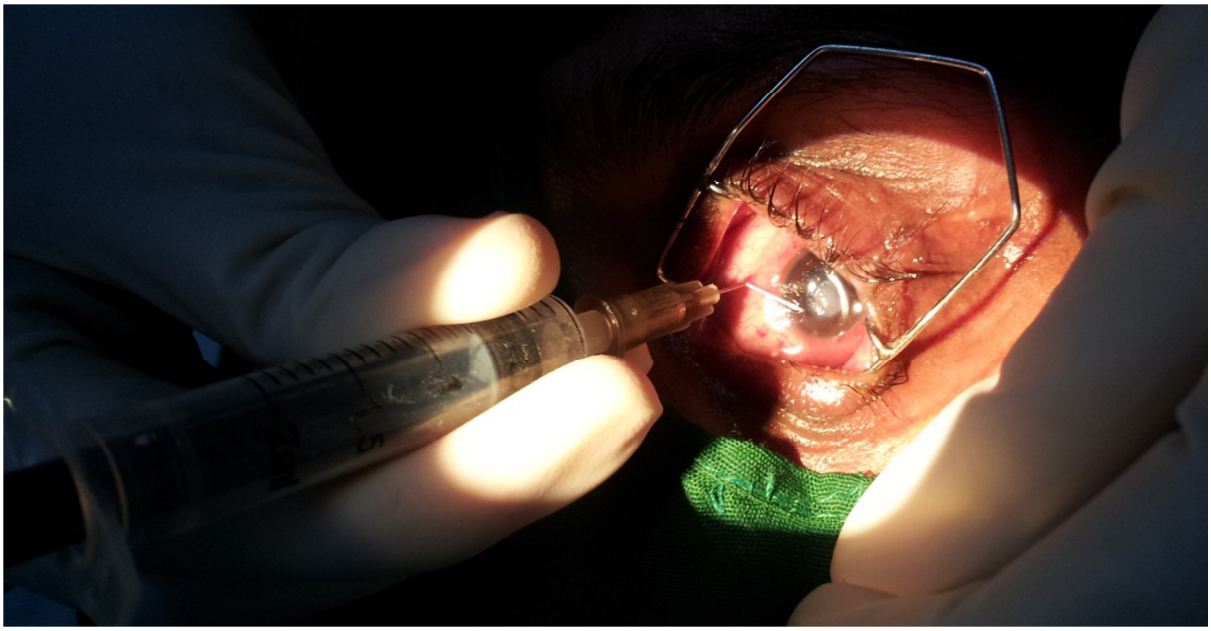
PERFORATED FUNGAL CORNEAL ULCER



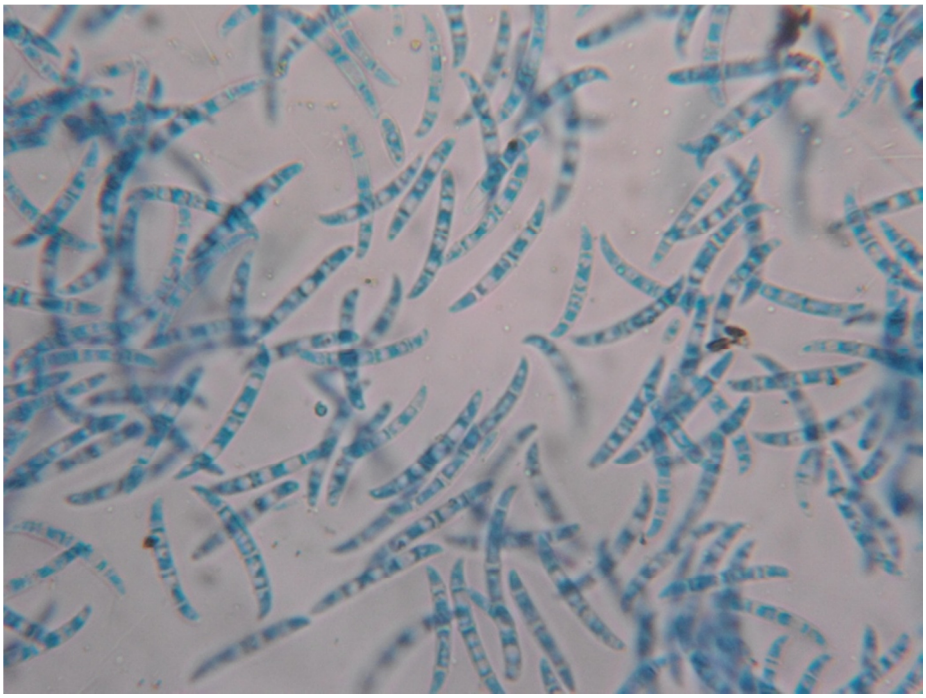
POST THERAPEUTIC PENETRATING KERATOPLASTY(patient no: 22)



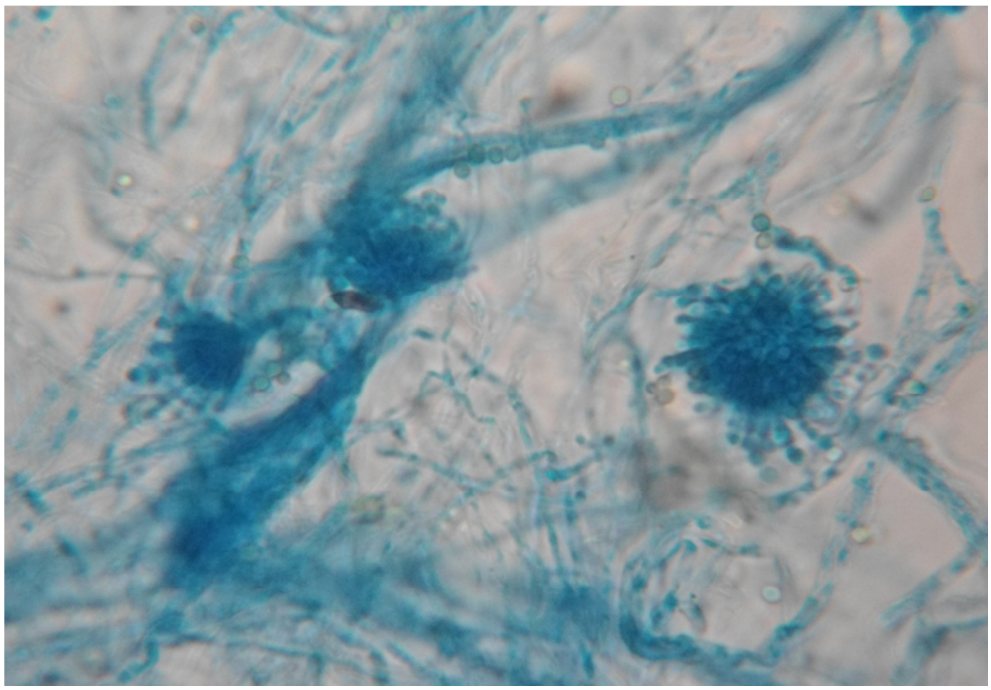
CASE OF PIGMENTED FUNGAL CORNEAL ULCER



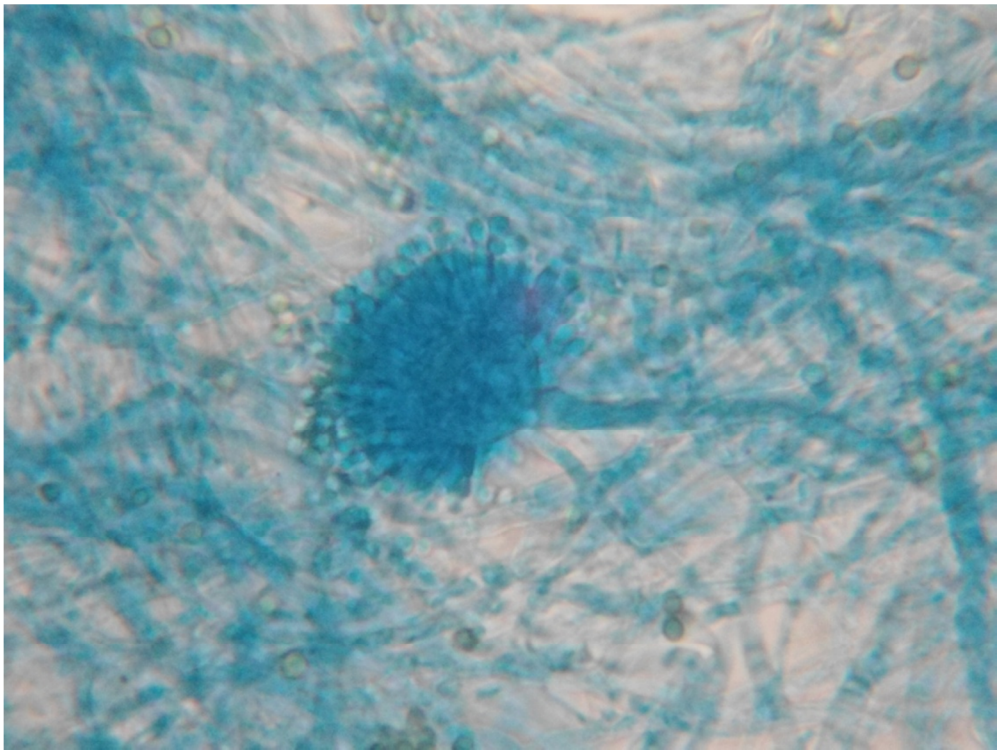
INTRASTROMAL VORICONAZOLE ADMINISTRATION UNDER MICROSCOPE



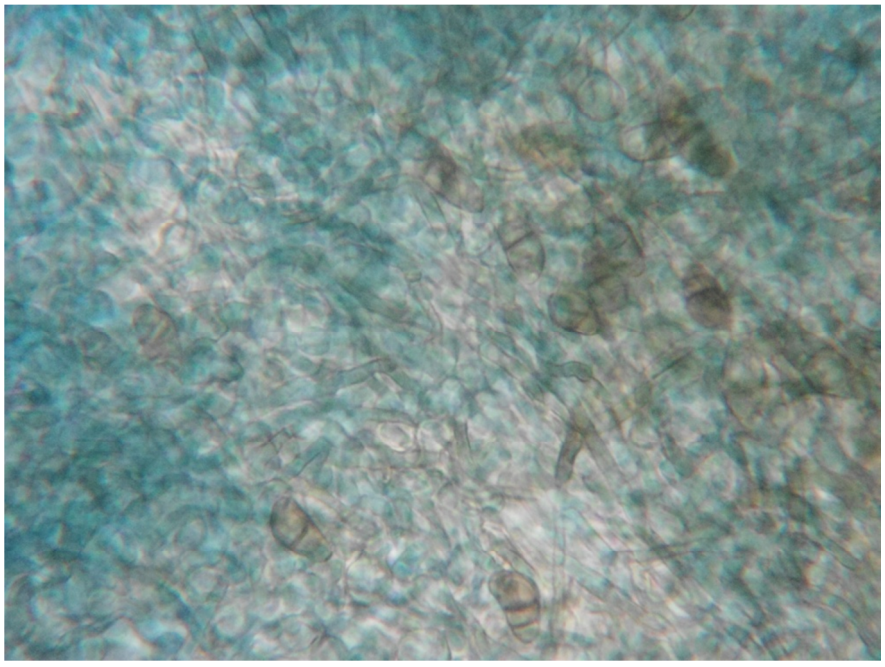
FUSARIUM SPECIES



ASPERGILLUS FLAVUS SPECIES



ASPERGILLUS NIGER



CURVULARIA SPECIES

PART-3

BIBLIOGRAPHY

TEXTBOOKS:

1. Grayson's diseases of the cornea, fourth edition, 257-283
2. Krachmer- third edition, page 277-283
3. Smolin and thoft- THE CORNEA- third edition, 229-253
4. Sir Stewart duke elder- diseases of outer eye, part 1, vol 8, page 385- 397
5. Parson's diseases of the eye, 21st edition, page 188-193
6. Kaufman- The Cornea, 1988, 217- 271
7. David J apple , fifth edition, 66-67
8. Principles and practice of ophthalmology- peyman, vol one, page 294-298
9. Jakobie principles and practice of ophthalmology, second edition, volume two, page 906-913
10. Kanski clinical ophthalmology 7th edition, page 180-187

JOURNALS:

1. Dua HS, Faraj LA, Said DG, Gray T, Lowe J (September 2013). "Human corneal anatomy redefined: a novel pre-Descemet's layer (Dua's layer)". Ophthalmology 120 (9): 1778–85.
2. Thomas PA.Fungal infections of the cornea.Eye 2003;17:852-62

3. Birk, D.E; and Trelstad, R.L.Extracellular compartments in matrix morphoenesis: collagen fibril, bundle and lamellar formation by corneal fibroblasts.J.Cell Biol.99: 2024, 1984
4. Kenyon KR, inflammatory mechanisms in corneal ulceration. Trans Am Ophthalmol Soc 1985;83:610-63
5. Bharathi MJ, Ramakrishnan R, Vasu S et al: epidemiological characteristics and laboratory diagnosis of fungal keratitis.indian J Ophthalmol 2003; 51:315-21
6. Upadhyay MP, Smolin G et al: epidemiological characteristics, predisposing factors, and etiological diagnosis of corneal ulceration in Nepal. Am J Ophth1991; 111:92-9
7. Choudhary A, Singh K. Spectrum of fungal keratitis in north india. Cornea 2005; 24; 8-15
8. Mselle J Fungal keratitis as an indicator of HIV infection in Africa. Trop Doct 1999;29(3): 133-135
9. Garg P,Gopinathan U, Chudhry K et al . keratomycosis : clinical and microbiological experience with dematiaceous fungi.Ophthalmology 2000; 107:574-580
10. Tuli SS et al, 2002 and Periman LM et al.,200

11. Wilhelmus KR,et al .fungal keratitis in contact lens wearers. Am J Ophthalmol 1998; 106: 708-14
12. Driebe et al: Microbial keratitis following cornea transplantation. Cornea 198; 2:41-5
13. Verma s, tuft sj. Fusarium solani keratitis following LASIK for myopia. Br J Ophthalmol 2002; 86:1190-91
14. Kaufman, H.E.and Wood, R.M. Mycotic keratitis.Am J Ophthalmol.59:993,1965
15. Forster RK, Wirta MG. Methanamine silver stained corneal scrapings in keratomycosis. Am J Ophthalmol 1976;82:261-5
16. Gopinathan U, Garg P et al: the epidemiological features and laboratory results of fungal keratitis. A 10 year review at a referral eye care centre in South India.Cornea 2002;21:555-9
17. Reis A, Sundmacher R, Tintelnot K, Agostini H, Jensen HE, Althaus C. Successful treatment of ocular invasive mould infection (fusariosis) with the new antifungal agent voriconazole. Br. J. Ophthalmol. 2000 Aug;84(8):932-3.
18. Troke P, Obenga G, Gaujoux T, et al. The efficacy of voriconazole in 24 ocular Fusarium infections. Infection. Published online 21 June 2012

19. Sharma S, Gopinathan U,Agarwal V: Early diagnosis of mycotic keratitis: predictive value of potassium hydroxide preparation. Indian J Ophthalmol 1998;46:31-5

20. Kumar M,Mishra NK, Shukla PK. Sensitive and rapid polymerase chain reaction based diagnosis of mycotic keratitis through single stranded conformation polymorphism. Am J Ophthalmol 2005;140(5):851-7

21. Das S,Samant M, Garg P, Vemuganti GK. Role of confocal microscopy in deep fungal keratitis.Cornea 2009;28(1):11-3

22. Forster RK.The role of excisional keratoplasty in microbial keratitis. In Cavanagh HD, ed.the cornea transactions of the world congress on the cornea 111.New York: Raven, 1988:529-533

23. O'Day et al: influence of corticosteroids invest ophthalmol vis sci 1984; 25:331

24. H Siatiri, F Daneshgar, N Siatiri, A Khodabande – The effects of intrastromal voriconazole injection and topical voriconazole in the treatment of recalcitrant fusarium keratitis. Cornea, 2011 – LWW

25. Sharma N, Agarwal P, Sinha R, Titiyal JS, Velpandian T, Vajpayee RB. Evaluation of intrastromal voriconazole injection in recalcitrant deep fungal keratitis: case series. Br J ophthalmol. 2011; 95(12): 1735-7.

26. Comparative evaluation of topical versus intrastromal voriconazole as an adjunct to natamycin in recalcitrant fungal keratitis. Sharma N, Chacko J, Velpandian T,Titiyal, Satpathy G, Tandon R, Vajpayee RB Ophthalmology. 2013 Apr; 120(4):677-81

27. Torres h et al: posaconazole : a broad spectrum triazole antifungal. Lancet infect dis. 2005: 5(12): 775-785

28. FlorCruz NV, Peczon IV, Evans JR. Medical interventions for fungal keratitis. Cochrane Database Syst Rev 2012;2:CD004241

29. Prajna NV, Mascarenhas J, Krishnan T, Reddy PR, Prajna L, Srinivasan M, et al. Comparison of natamycin and voriconazole for the treatment of fungal keratitis. Arch Ophthalmol. 2010;**128**(6):672-8.

30. Prajna NV, Krishnan T, Mascarenhas J, et al. The mycotic ulcer treatment trial: A randomized trial comparing natamycin vs voriconazole. JAMA Ophthalmol 2013;131:422-9.

31. Hariprasad SM, Mieler WF, Lin TK, Sponsel WE, Graybill JR. Voriconazole in the treatment of fungal eye infections: a review of current literature. Br J Ophthalmol. 2008;**92**(7):871-8.

32. Freda R. Use of oral voriconazole as adjunctive treatment of severe corneal fungal infection: case report. Arq Bras Oftalmol 2006;69:431– 434.

33. Bunya VY, Hammersmith KM, Rapuano CJ. Topical and oral voriconazole in the treatment of fungal keratitis. Am J Ophthalmol. 2007;143:151–3.

34. Lalitha P, Shapiro BL, Srinivasan M, Prajna NV, Acharya NR, Fothergill AW, et al. Antimicrobial susceptibility of Fusarium, Aspergillus, and other filamentous fungi isolated from keratitis. Arch Ophthalmol. 2007;125:789

35. The Epidemiological Features and Laboratory Results of Fungal Keratitis: A 10-Year Review at a Referral Eye Care Center in South India

36. Gopinathan, Usha Ph.D.; Garg, Prashant M.S.; Fernandes, Merle M.S.; Sharma, Savitri M.D.; Athmanathan, Sreedharan M.D.; Rao, Gullapalli N. M.

37. **Gonzales CA**, Srinivasan M, Whitcher JP, *et al*. Incidence of corneal ulceration in Madurai District, south India. Ophthalmic Epidemiol 1996;**3**:159–66.

38. **Whitcher JP,** Srinivasan M, Upadhyay MP. Corneal blindness: a global perspective. Bull World Health Organ 2001;**79**:214–21.

39. Bashir G, Shah A, Thokar MA, Rashid S, Shakeel S. Bacterial and fungal profile of corneal ulcers–a prospective study. Indian J Pathol Microbiol 2005. Apr;48(2):273-277

40. Upadhyay MP, Karmacharya PC, Koirala S, Tuladhar NR, Bryan LE, Smolin G, et al. Epidemiologic characteristics, predisposing factors, and etiologic diagnosis of corneal ulceration in Nepal. Am J Ophthalmol 1991. Jan;111(1):92-99

41. Laspina F, Samudio M, Cibilis D, Ta CN, Farina N, Sanabria R, et al. Graefes Epidemiological characteristics of microbiological results on patients with infectious corneal ulcers: A 13 year survey in Paraguay. Arch Clin Exp Ophthalmology 2004;242:204-209 .

42. Leck AK, Thomas PA, Hagan M, Kaliamurthy J, Ackuaku E, John M, et al. Etiology of supportive corneal ulcers in Ghana and south India and epidemiology of fungal keratitis. BJO 2002;86:1211-1215 .

PROFORMA

NAME : OCCUPATION :
CCNO :

AGE : OPNO/IPNO :

SEX : DOA :

C/O PAIN/IRRITATION/REDNESS/WATERING

DURATION:

EYE:

H/O TRAUMA:

PAST H/O:DM/HT/TB/ASTHMA/ORGAN TRANSPLANTATION/HIV

H/O CONTACT LENS USE

H/O DRUG INTAKE

PERSONAL H/O:H/O SMOKING

OCCUPATIONAL HISTORY:

GENERAL EXAMINATION

EXAMINATION OF THE EYES RE LE

VISION

LIDS

OCULAR MOVEMENTS

CONJUCTIVA

CORNEA – SIZE OF ULCER

DEPTH

IRIS

ANTERIOR CHAMBER

PUPILS

LENS

TENSION

EXAMINATION AFTER STAINING WITH FLUORESCEIN :

INVESTIGATIONS

BE DUCT

TENSION

FUNDUS

RBS

URINE ALB /SUGAR

CORNEAL SMEAR:

- KOH MOUNT
- GRAM STAIN
- CULTURE

INTRASTROMAL VORICONAZOLE GIVEN ON:

FOLLOW UP

S.NO	NAME	CORNEA CLINIC NO	AGE(YEARS)	SEX	OCCUPATION	EYE	H/O INJURY	INITIAL VISUAL ACUITY	SIZE OF ULCER	DEPTH OF ULCER	HYPOPYON	satellite	immun ring	KOH MOUNT	FUNGAL CULTURE	response	FINAL VISUAL ACUIT	final size of ulcer
1	Krishnan	13951	60	M	FARMER	RE	vegetable matter	1/2/60 NIP	4*4	1/3rd	+	-	-	POSITIVE	asp. Fumigatus	GOOD-HEALED	4/60 nip	3*3
2	Nathan	14469	48	M	-	RE	-	1/60 nip	4*5	1/3rd	+	-	-	POSITIVE	Fusarium	GOOD-HEALED	6/60 p	3*2
3	Devaki	14975	47	F	housewife	LE	vegetable matter	cfcf	6*5	2/3rd	+	+	+	POSITIVE	asp.niger	tkp	hm+	7*6
4	Bayammal	15667	48	F	housewife	LE	vegetable matter	1 /60 nip	4*4	1/3rd	-	-	-	POSITIVE	asp. Fumigatus	good-healed	1/60 nip	3*2
5	Narasaiyah	14500	41	M	FARMER	LE	with thorn	6/60 nip	6*4	2/3rd	+	+	-	POSITIVE	asp.flavus	tkp	1/60nip	7*7
6	Rajendran	14557	30	M	labourer	RE	foreign body	CFCF	3*3	1/3rd	+	-	-	POSITIVE	Fusarium	GOOD-HEALED	6/60 nip	3*2
7	gayathri	14118	16	F	student	re	wooden stick	HM+	5*4	1/3rd	+	-	-	POSITIVE	asp. Fumigatus	GOOD-HEALED	6/36 p	4*3
8	Bhaskar	15406	42	M	FARMER	RE	foreign body	1/ 60 nip	3*3	1/3rd	+	-	-	POSITIVE	asp.niger	GOOD-HEALED	6/60 nip	2*2
9	Pavadai	13998	52	M	FARMER	LE	wooden stick	CFCF	5*5	2/3rd	+	-	-	POSITIVE	asp.flavus	TKP	pl+	6*6
10	Chellamal	18015	55	F	labourer	RE	-	2/60 NIP	4*4	1/3rd	+	-	-	POSITIVE	asp.fumigatus	GOOD-HEALED	HM+	3*3
11	egambaram	18652	42	M	-	RE	-	6/60 nip	4*3	1/3rd	+	+	-	POSITIVE	Fusarium	GOOD-HEALED	6/60 with ph 6/36	3*2
12	Dinakaran	17654	51	M	FARMER	LE	vegetable matter	1/60 nip	5*6	2/3rd	+	-	-	POSITIVE	asp.flavus	tkp	hm+	7*6
13	Arul	17190	35	M	-	LE	wooden stick	6/60 nip	4*4	2/3rd	-	-	-	POSITIVE	asp.niger	GOOD-HEALED	6/36 nip	3*3
14	Syed Basha	17214	27	M	labourer	LE	vegetable matter	5/60 nip	3*3	1/3rd	-	-	-	POSITIVE	ZYGOMYCES SP.	GOOD-HEALED	6/60 nip	2*3
15	Ayyappan	17218	30	M	labourer	RE	foreign body	HM+	4*2	1/3rd	-	-	-	POSITIVE	asp. Fumigatus	GOOD-HEALED	1/60 nip	3*2
16	Selvi	17107	50	F	FARMER	RE	wooden stick	HM+	5*5	2/3rd	+	-	-	POSITIVE	asp.niger	TKP	PL+	7*6
17	Yuvaraj	16518	44	M	FARMER	LE	vegetable matter	5/ 60 nip	4*5	2/rd	+	-	-	POSITIVE	asp. Fumigatus	TKP	HM+	7*6
18	Akilan	16458	39	M	labourer	LE	vegetable matter	1/60 nip	4*4	1/3rd	-	-	-	POSITIVE	asp.niger	GOOD-HEALED	6/18p	3*2
19	Kanniammal	16293	65	F	housewife	RE	foreign body	2/60 NIP	4*4	1/3rd	+	-	-	POSITIVE	Fusarium	GOOD-HEALED	6/60 nip	3*3
20	Sampath	16196	24	M	FARMER	LE	-	1/60 nip	5*5	2/3rd	+	-	-	POSITIVE	Fusarium	tkp	hm+	7*7
21	Sekar	16109	54	M	FARMER	RE	wooden stick	3/60 with PH 6/60	3*5	2/3rd	+	+	+	POSITIVE	asp. Fumigatus	GOOD-HEALED	6/36 nip	3*3
22	Vasantha	16542	45	F	FARMER	RE	foreign body	HM+	4*6	2/3rd	+	-	-	POSITIVE	asp. Fumigatus	TKP	HM+	7*6
23	kanniappan	15622	57	M	FARMER	LE	wooden stick	1/60 NIP	4*3	1/3rd	+	-	-	POSITIVE	asp.niger	GOOD-HEALED	1/60 NIP	3*3
24	Lakshmi	15838	27	F	housewife	LE	foreign body	4/60 nip	3*3	1/3rd	-	-	-	POSITIVE	asp.flavus	GOOD-HEALED	6/60 with ph 6/36	3*2
25	Mohammad	155451	31	M	labourer	LE	vegetable matter	3/60 NIP	3*4	2/3rd	+	-	-	POSITIVE	Fusarium	GOOD-HEALED	6/60 NIP	2*3

KEY TO MASTER CHART

RE- RIGHT EYE

LE- LEFT EYE

TKP- THERAPEUTIC PENETRATING KERATOPLASTY

ASP - ASPERGILLUS SPECIES